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Allelic variation analysis and development of gene-specific molecular markers confering acid soil tolerance in barley (*Hordeum vulgare* L.)

by

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Publications

Bian M, Waters I, Broughton S, Zhang X-Q, Zhou M, Lance R, Sun D, Li C (2013) Development of gene-specific markers for acid soil/aluminium tolerance in barley (*Hordeum vulgare L.*). Mol Breed 32 (1):155–164 (Chapter 2)

Bian M, Zhou M, Sun D, Li C (2013) Molecular Approaches Unravel the Mechanism of Acid Soil Tolerance in Plants. Crop J 1 (2):91–104 (Chapter 1)

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Bian M, Walters I, Broughton S, Sun D, Zhou M and Li C (2011) A gene-specific PCR marker for detecting acid soil tolerance in barley *(Hordeum vulgare* L.*)*, Proceedings of the 15th Australian Barley Technical Symposium: Barley for Business, Adelaide, South Australia (Chapter 2)

Bian M, Walters I, Broughton S, Su D, Shabala S, Zhou M and Li C (2013) A gene-specific SNP marker for acid soil and aluminum toxicity tolerance in barley. Crop Pasture Sci (In preparation) (Chapter 3)

Bian M, Walters I, Broughton S, Su D, Shabala S, Zhou M and Li C (2013) Allelic variations and association mapping analysis of Al tolerance based on *HvAACT1* gene in barley (*Hordeum vulgare*. L). PLoS One (In preparation) (Chapter 4)

Abstract

Acid soil is a prevalent problem over the world. The high concentration of Al in the acid soil is one of the major production limiting factors to many plants. Decades of studies have resulted in a significant progress in revealing the mechanism of Al tolerance in plants. Several key genes have been identified and the Al tolerance was validated to be related with gene sequence variations in some plants. Barley (*Hordeum vulgare* L.) is one of most sensitive cereals to aluminum toxicity. This thesis was aimed at revealing the mechanisms of Al tolerance in barley using the marker development, QTL, association mapping and sequencing techniques. The major findings of the thesis are listed below:

1. The genetics of barley Al tolerance was studied in two double haploid populations, Hamelin/Svanhals and Br2/Hamelin, through QTL mapping. The phenotypic vitiation was investigated in both hydroponic and acid soil experiments. The phenotypic result suggested that a single gene is responsible for Al tolerance in barley. Al tolerance is controlled by single QTL on chromosome 4H and flanked by commonly used SSR makers.

2. Gene-specific markers were developed covering the whole sequence of *HvAACT1* gene (also named as *HvMATE*). The polymorphic gene-specific markers were incorporated with the other commonly used SSR markers to conduct the QTL mapping. The result showed that the QTL interval for acid soil tolerance was narrowed and the phenotypic variations explained by the QTLs were increased. The gene-specific markers could also explain more phenotype variation than these

commonly used SSR markers. These new gene-specific markers provide effective and simple molecular tools for marker assisted selection in acid soil tolerant barley breeding.

3. The genetic diversity analysis and candidate gene association mapping based on *HvAACT1* gene were conducted using accessions with different Al tolerance. Twenty eight gene-specific markers were polymorphic among different accessions. The sequencing analysis showed that these polymorphisms among accessions varied from over 1Kb to one SNP. These markers clearly identified the genetic relationship of different accessions using cluster analysis. Several gene specific markers were found to be associated with the Al tolerance in accessions. These significant polymorphisms detected by these markers could be considered candidate variation sites related to Al tolerance.

4. The allele variations of *HvALMT* were also studied in different accessions. Ten pairs of primers (6 in the coding region, 4 in the upstream region) showed polymorphisms. Sequencing analysis showed that these polymorphisms varied from one SNP to over 400bp insertion/deletion. Stepwise regression analysis revealed one gene-specific marker, UA21, was significantly correlated with the phenotypic variation of root length in acid soil and the relative root length in acid soil, as well as acid soil treated with lime.

In conclusion, the findings from this thesis suggest that: 1) While citrate exudation was validated to be responsible for Al tolerance in barley, it cannot fully explain the genetic variability in Al tolerance in barley; 2) Malate exudation may play an important role in detoxifying Al in some barley accessions; 3) The gene specific markers developed from *HvAACT1* and *HvALMT* can be used in molecular

marker assisted selection in breeding; and 4) The significant polymorphisms detected by the association mapping can be used for further gene expression study.

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List of abbreviations

AA	Amino acid
ABC	ATP-binding cassette
AFLP	Amplified Fragment Length Polymorphism
ALMT	Aluminum-activated malate transporter
Alp	Tolerance to aluminium and low pH
Alp3	Tolerance to aluminium and low pH 3
als3	Al sensitive 3
Alt	Aluminium tolerance
ANOVA	Analysis of variance
APS	Ammonium peroxosulphate
ART1	Al^{3+} resistance transcription factor 1
AtALMT1	*Arabidopsis thaliana* aluminum-activated malate transporter
AtBCB	*Arabidopsis thaliana* blue copper binding protein
AtMATE1	*Arabidopsis thaliana* multidrug and toxin extrusion
ATP	Adenosine triphosphate
BnALMT	*Brassica napus* aluminum-activated malate transporter
BnMATE	*Brassica napus* multidrug and toxin extrusion
bp	Base pair
CAPS	Cleaved amplified polymorphic sequences
clf-59	CURLY LEAF -59
CYMMIT	International Maize and Wheat Improvement Centre
DArT	Diversity Arrays Technology

DH	Double hiploid
DI	Deionized
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetraacetic Acid
EST	Expressed sequence tag
FLC	FLOWERING LOCUS C
GWAS	Genome Wide Association Studies
HvAACT	*Hordeum vulgare* Aluminum Activated Citrate Transporter
HvALMT	*Hordeum vulgare* Aluminum-activated malate transporter
HvMATE	*Hordeum vulgare* multidrug and toxin extrusion
indel	insertion–deletion
ISSRs	Inter-simple sequence repeats
Kb	Kilo base pair
LD	Linkage disequilibrium
MAS	Marker-assisted selection
MATE	multi-drug and toxic compound extrusion
MITEs	Miniature inverted repeat transposable elements
MLM	Mixed linear model
NILs	Near isogenic lines
Nramp	Natural resistance-associated macrophage protein
Nramp	Natural resistance-associated macrophage protein
Nrat1	Nramp aluminum transporter 1
NtGDI1	*Nicotiana tabacum* GDP-dissociation inhibitor
NtPOX	*Nicotiana tabacum* peroxidase
NtSTOP1	*Nicotiana tabacum* sensitive to proton rhizotoxicity

OA	Organic acid
PAGE	Polyacrylamide gel electrophoresis
parB	Tobacco glutathione S-transferase
PCR	Polymerase chain reaction
pht	Tolerance to low pH
PIC	Polymorphism information content
QTL	Quantitative trait loci
RAPD	Randomly Amplified of Polymorphic DNA
REML	Restricted maximum likelihood
RFLP	Restriction Fragment Length Polymorphism
RNAi	RNA interference
rpm	Revolutions per minute
RT-PCR	Reverse transcription-polymerase chain reaction
SbMATE	*Sorghum bicolor* Multidrug and Toxin Extrusion
ScAACT1	*Secale cereale* aluminum-activated citrate transporter
ScALMT1	*Secale cereale* aluminum-activated malate transporter
SCARs	sequence characterized regions
SNP	Single nucleotide polymorphisms
SSCP	Single-strand conformation polymorphism
SSR	simple sequence repeats
STAR1	Sensitive to Al rhizotoxicity 1
STOP1	Sensitive to proton rhizotoxicity
STS	Sequence tag site
SV	Slow vacuolar
TaALMT1	*Triticum aestivum* Aluminum-activated malate transporter

TBE Tris-borate-ethylenediaminetetraacetic acid

TEMED Tetramethylethylenediamine

UPGMA Unweighted pair-group method with arithmetic average

WAK1 Cell wall-associated receptor kinase 1

ZmMATE1 *Zea mays* Multidrug and Toxin Extrusion 1

ZmMATE2 *Zea mays* Multidrug and Toxin Extrusion 2

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Introduction

Acid soils cause plant production loss all over the world (Von Uexkuell and Mutert 1995). Detrimental effects of soil acidity are a combination of increased solubility of metal ions (especially Al^{3+}), low pH solution and a reduced availability of essential nutrients (Foy et al. 1978). Exogenous application of lime (Dolling et al. 1991), boron (Yu et al. 2009), and magnesium (Silva et al. 2001) was reported to partially ameliorate acid soil toxicity. However, given the limitation of this approach (e.g. environmental aspects and cost; Bian et al. 2013); breeding tolerant cultivars appears to be much more promising way to deal with soil acidity. Plant species vary differently in their tolerance to acid soils. Decades of physiological and genetic studies revealed that the external mechanism based on organic acid exudation is mainly responding for the acid soil tolerance in plants (Wang et al. 2006b; Kochian et al. 2004). Owing to the development of the molecular approaches, several responsible genes and QTLs were identified in some species. These genes mainly belong either to MATE or ALMT gene family responding for citric acid or malic acid exudation, respectively (Delhaize et al. 2007). Also, different types of molecular markers including DArT (Stodart et al. 2007), SSR (Raman et al. 2003; Wang et al. 2006a), STS (Wang et al. 2007), RFLP (Riede and Anderson 1996), AFLP (Miftahudin et al. 2002) and gene-specific markers (Raman et al. 2008) were developed to be used in molecular assisted selection in breeding. Of them, the gene-specific markers were much more preferred for their directly tracing of interesting genes.

Barley, which is the fourth most important cereal in the world (Dai et al. 2012),

was validated to be the most sensitive cereals to soil acidity, which results in severe limitation to its production and distribution. Two genes were found to be responsible for organic acid extrusion in barely. *HvAACT1*, located on chromosome 4H, is responding for citric acid exudation (Furukawa et al. 2007), and *HvALMT*, located on chromosome 2H, is responsible for the malic acid exudation (Gruber et al. 2010). *HvAACT1* is the most well studied gene, and has been considered by many researchers as the major gene conferring Al tolerance in barley (Fujii et al. 2012; Furukawa et al. 2007). *HvALMT* has been cloned and validated to be a malate transporter, but there was no report on its relationship with Al tolerance (Gruber et al. 2011).

Barley genotypes vary greatly in the acid soil tolerance. Currently, most previous research attributed the multiple levels of Al tolerance between genotypes to the sequence variation of the responding genes. Examples include the repeat region in wheat *TaALMT1* gene (Sasaki et al. 2006), MITEs in sorghum *SbMATE* gene (Magalhaes et al. 2007), 1kb indel in *HvAACT1* gene, and the multiple copies of *ZmALMT1* gene (Fujii et al. 2012; Maron et al. 2013).

Although the above two genes in barley have been sequenced and cloned, their respective roles (and, hence, mechanisms of Al tolerance in barely) are still poorly understood, which limits barley breeding for Al tolerance. While most studies suggested that one major gene on chromosome 4H was responsible for the Al tolerance in barley, this view was challenged by some conflicting reports (Tang et al. 2000; Navakode et al. 2009). In many other plant species, such as wheat (Ryan et al. 2009; Sasaki et al. 2004), *Secale cereal* (Silva-Navas et al. 2011; Collins et al. 2008) and *Arabidopsis* (Hoekenga et al. 2006; Liu et al. 2009), both MATE and ALMT

genes were found to be responsible for Al tolerance. However, in barley, only *HvAACT1* gene was considered to be important to confer Al tolerance, while the role of another gene, *HvALMT*, was envisaged in maintaining anion homeostasis in the cytosol and osmotic adjustment (Gruber et al. 2011; Gruber et al. 2010). To the best of my knowledge, no direct evidence exists for the role of *HvALMT* in Al tolerance in barley. However, considering other plant examples, the possibility cannot be neglected.

Different types of molecular markers have been developed and used to trace interesting genes/loci conferring plant acid soil tolerance; of these, gene-specific markers are more preferred by breeders. However, until now, no gene-specific marker was available for either *HvAACT1* or *HvALMT* in barley.

To address the above issues and fill the existing gaps in our knowledge, the following the aims were set up: first, to identify new genes responding for Al tolerance in barley (Chapter 2 and Chapter 3); second, to search for new alleles for both citrate transporter and malate transporter genes (Chapter 4 and Chapter 5); third, to better understand sequence variation of *HvAACT1* and *HvALMT* genes associated with phenotypic variation (Chapter 4 and Chapter 5); and finally, to develop gene-specific markers as molecular tools for marker-assisted selection for acid soil tolerance breeding in barley (Chapter 2, Chapter 3, Chapter 4 and Chapter 5).

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Chapter 1

Literature Review

Molecular Approaches Unravel the Mechanism of Acid Soil Tolerance in Plants

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Chapter 2

Development of gene-specific markers for acid soil/aluminium tolerance in barley (*Hordeum vulgare* L.)

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Chapter 3 - A gene-specific SNP marker for acid soil and aluminium toxicity tolerance in barley

This chapter will be submitted to Crop and Pasture Science:

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A gene-specific SNP marker for acid soil and aluminum toxicity tolerance in barley. Crop Pasture Sci (In preparation)

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3.1 Introduction

Al tolerance related genes in plants involved in multiple metabolism process including cell elongation and division, cell wall formation, oxidative stress, iron metabolism, signal transduction and other cellular mechanisms (Inostroza-Blancheteau et al. 2011; Eticha et al. 2010; Maron et al. 2008). It is reported that the expression of several Al-induced genes, such as AtBCB, parB, NtPOX and NtGDI1 in transgenic *Arabidopsis* plants showed better relative root growth under Al stress (Ezaki et al. 2000). The secretion of organic anions such as citrate, malate from the root apices plays an important role in excluding and detoxifying Al (Ma et al. 2001; Singh and Chauhan 2011; Kochian et al. 2004). Among many Al-induced genes, ALMT and MATE genes responsible for malic acid and citric acid extrusion, respectively, were reported to be conferring Al tolerance in many plants (Yang et al. 2012).

Barley is one of the most sensitive species to Al toxicity among small-grain crops (Foy et al. 1978; Zhao et al. 2003) but differences in Al tolerance exist among varieties. Barley Al tolerance was mainly controlled by one single gene but several QTLs were also reported (Wang et al. 2006; Navakode et al. 2009; Bian et al. 2013). The major tolerance gene on chromosome 4H was given various names according to its origin or the tolerance to Al^{3+} toxicity or low pH, which include *Pht* (low soil pH) (StøLen and Andersen 1978), *Alp* (Al tolerance gene in Dayton) (Minella and Sorrells 1997), *Alt* (Al tolerance gene in WB229) and *Alp3* (Al tolerance gene in Brindabella) (Raman 2001; Raman et al. 2002a). Ma et al (2004) reported a QTL linked tightly with aluminum tolerance explained more than 50% of the phenotypic variation in citrate secretion in a cross between an Al-resistant cultivar

(Murasakimochi) and an Al-sensitive cultivar (Morex). This QTL was located at the same position of the major tolerance gene with marker Bmag353 being tightly linked marker (Ma et al. 2004). Fine mapping combined with microarray analysis identified that *HvAACT1*, a MATE gene (also known as *HvMATE*), was responsible for the Al-activated citrate secretion (Furukawa et al. 2007). Heterologous expression of *HvAACT1* in Xenopus oocytes showed efflux activity for ^{14}C-labeled citrate and overexpression of this gene in tobacco showed increased Al tolerance (Furukawa et al. 2007). Further study demonstrated that relative expression of the *HvMATE* gene in the *Alp* locus on chromosome 4H was 30-fold higher in Dayton (tolerant) than Gardiner (susceptible). The expression marker exhibited complete linkage with the *Alp* locus in the DH population accounting for 72% of the variation for Al tolerance based on relative root growth under Al^{3+} stress (Wang et al. 2007). These results further supported the notion that *HvMATE*, a gene encoding a multidrug and toxic compound extrusion protein, is the candidate gene controlling Al tolerance on chromosome 4H.

Our previous study on a double haploid population from the cross of Hamelin/Svanhals also confirmed that the tolerance was controlled by a single gene on chromosome 4H. However, several genes were reported in wheat for Al tolerance. The first gene, *TaALMT1*, controlling malate extrusion co-segregated with Al tolerance in an F_2 population derived from a cross between ET7 and ES7 and F_3 populations derived from a cross between ET8 and ES8 (Sasaki et al. 2004). The second gene, *TaMATE*, responding for citrate extrusion was identified in an F_2 population derived from a cross between Carazinho, a Brazilian wheat cultivar, and EGA-Burke.

In a present study, another population derived from a cross between Br2, a

Brazilian barley cultivar, and Hamelin was used to study the genetics of Al tolerance in barley. Another gene-specific molecular marker was developed from *HvAACT1* gene sequence and was validated in the Hamelin/Br2 double haploid population. Results showed that the phenotype variation explained by the new gene specific marker was much more than that by currently used markers. The changes of amino acids revealed by the marker covered polymorphic region may be related to phenotypic variation in Al tolerance.

3.2 Materials and methods

3.2.1 Plant material

A double haploid population derived from a cross of Hamelin/Br2 was generated by a standard method of anther culture (Jähne-Gärtner and Lörz 1999). This population consisted of 158 lines. The female parent cultivar Hamelin from Western Australian barley breeding program is Al sensitive (see reference from http://www.agric.wa.gov.au/objtwr/imported_assets/content/fcp/cer/bar/cp/fn2005_hamelin.pdf) and the male parent Br2 from Brazil showed very good tolerance to acid soil (see reference from http://www.ausgrain.com.au/Back%20Issues/184ndgrn08/36_Mutation.pdf).

Additional 56 accessions which differ significantly in acid soil/Al tolerance were collected from different parts of the world for the validation of the gene-specific molecular markers (Appendix Table A-1).

3.2.2 Phenotypic measurements

Soil assay: Acid soil was collected from the 10–30 cm layer from Merredin WA. Soil pH was 4.2 with soluble aluminium of 8.1 mg/kg. For the control

treatment, lime was added to the same soil to adjust the pH to 6.5. Five seeds of each line were sown in pots containing acid or limed acid soil. The seedlings were removed from the soil for root length measurements one week after sowing. Root length was used as the parameter for acid soil tolerance. The experiment was conducted in a glasshouse and each experiment was repeated three times.

Hydroponic methods: Three hydroponic treatments were used to screen barley for acid/aluminum tolerance; i) control treatment at pH 6.5; ii) acid treatment at pH 4.2 and iii) acid + aluminium treatment at pH 4.2 with 2 ppm aluminium. All three treatments contained the same nutrient solution with the following macronutrients (mM): $CaCl_2 \cdot 2H_2O$, 4.0; $(NH_4)_2SO_4$, 0.1; KNO_3, 6.5; $MgCl_2 \cdot 6H_2O$, 2.5; NH_4NO_3, 0.4 and the following micronutrients (μM): NaH_2PO_4, 13; $MnSO_4 \cdot H_2O$, 2; $CuSO_4 \cdot 5H_2O$, 0.3; $ZnSO_4 \cdot 7H_2O$, 0.8; H_3BO_3, 10; $Na_2MoO_4.2H_2O$, 0.1 and $FeSO_4 \cdot .7H_2O$, 10. In the acid + aluminium treatment, Al was included as $AlCl_3 \cdot 6H_20$ at 74 μM (2 ppm Al). Approximately 50 seeds of each line were placed in a 9 cm Petri-dish with 5 ml DI water. Extra seeds of each line were included (approximately double) to ensure that there were enough seeds with similar root lengths for the experiment. The Petri-dishes were wrapped in Clingfilm in bundles of 10-20 and the seeds were incubated in the dark for 45 hours at 4ºC, then 47-48 hours at 18ºC. Germinated seeds were placed into allocated positions on the grid trays ensuring that roots were kept moist during the process. The seeds were positioned so that roots were facing downwards into the nutrient solution. Seeds with root lengths between 4-7 mm were selected preferentially. Replicate 1 was sown on the first day and Replicate 2 was sown on the second day. Germinated seeds were stored at 4ºC between sowing the two replicates. The grid trays containing germinated seeds were placed into three treatment solutions and grown

at 20/15ºC (day/night) in a controlled environment room with a 12-hr daylength. All solutions were bubbled gently with air stones and aquarium pumps to aerate the solutions and prevent stagnation. The pH of the solutions was checked daily and adjusted as necessary using either KOH or HCl. Dosing meters, which dosed acid or alkali, were also used to maintain pH at desired levels. Root lengths were measured after 7 days and eight traits were recorded: Acidsoil, pH42, pH65, pH42Al, RL42, RL42Al, AvAv and ALT (Table 3-1).

Table 3-1 Traits and their meanings, MLW stands for Mean root length of whole population

Trait	Meaning
Acidsoil	Mean root length under acid soil (mm)
pH42	Mean root length under pH4.2 (mm)
pH42Al	Mean root length under pH4.2+Al (mm)
RL42	pH42/pH65
RL42Al	pH42Al/pH65
pH65	Mean root length under pH6.5 (mm)
AvAv	((pH42Al/MLW pH4.2+Al) + (Acidsoil/MLW acid soil))/2
ALT	Al tolerance grouped using the phenotypic data from acid soil and pH4.2+Al treatment

3.2.3 Primer derivation and Genotyping of the DH population

Thirty-seven DH lines from the population, representing typical tolerant or sensitive individuals in the population based on phenotype, were selected and genotyped using Diversity Arrays Technology (DArT) (http://www.diversityarrays.com). In addition, 446 commonly used SSR markers were synthesized using information from previous publications (Maroof et al. 1994;

Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998; Cardle et al. 2000; Pillen et al. 2000; Ramsay et al. 2000; Li et al. 2003; Thiel et al. 2003; Rostoks et al. 2005; Varshney et al. 2007) and used for screening polymorphic markers in all 158 DH lines of this population.

Forty-four primers were synthesized from contig_51011 containing the *HvAACT1* gene from the International Barley Genome Sequencing Consortium (http://webblast.ipk-gatersleben.de/barley/viroblast.php) using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA). The details of primers were listed in see Chapter 2 Fig. 2.5.

3.2.4 DNA extraction, PCR reaction and sequencing

Young leaves from plant material of 2-week old seedlings were cut by scissors and stored at -80ºC. DNA was extracted from the leaves using the phenol/chloroform method (Yang et al. 2009) with a slight modification.

The products were loaded on 2% agarose, 6% polyacrylamide or 12% SSCP/TBE gels, stained with ethidium bromide and visualized under UV light to detect polymorphisms in different cultivars. The SSCP method followed previously described procedures (Savov et al. 1992; Martins-Lopes et al. 2001) (acrylamide/bisacrylamide ratio of 37.5:1) in cold 0.5 × TBE and run at room temperature for 24 to 36 h. Direct sequencing reaction was conducted after the PCR product was purified using a QIAquick PCR purification kit (Qiagen). PCR products were sequenced directly in both directions using the Big-Dye^{TM} Terminator method on an Applied Biosystems 3730 DNA Sequencer (SABC, Murdoch University, Western Australia).

3.2.5 Data analysis

Six phenotypic traits including Acidsoil, pH42, pH42Al, RL42, RL42Al and AvAv (Table 3-1) were used to conduct the QTL analysis. Association between the markers and traits was calculated by PASW Statistics v.18 (SPSS Inc., Chicago, USA). Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) was used to design all the gene specific primers. Geneious software package Geneious v5.1, available from (http://www.geneious.com.) and BioEdit (http://www.mbio.ncsu.edu/BioEdit) with default parameters were used for DNA sequence editing, comparison and alignment. Linkage map construction and QTL analysis were performed using QTL IciMapping Version 3.2 at LOD=3.0 and Kosambi map unit function (Kosambi 1943; Manly 1993; Li et al. 2007; Li et al. 2010a).

3.3 Results

3.3.1 Phenotyping and inheritance of Al tolerance in barley

The Hamelin/Br2 DH population was assessed using acid soil-grown plants and hydroponic experiments including three treatments: pH4.2, pH4.2 + Al, and pH6.5. The two parents showed significant difference in Al tolerance. Under acid soil treatment, the average root length of the female parent Hamelin was 123 ± 40.2 mm, while that of the male parent Br2 was 191 ± 44.7 mm. In hydroponic experiments, the two parents showed no significant (at $P < 0.05$) difference in root growth under pH6.5 treatment, with the average root length of Hamelin and Br2 being 88 ± 10.8 and 92 ± 6.5 mm, respectively. When the pH of the solution was reduced to 4.2, the root growth of both varieties was significantly inhibited with the root length of the two varieties being reduced to 47 ± 3 and 41 ± 7.1 mm, respectively. Further

addition of Al to pH4.2 solution significantly reduced the root growth of the sensitive cultivar but showed little effects on the tolerant cultivar. Under pH4.2+Al treatment, the average root length of Hamelin was 22 ± 2.1 mm, a reduction of more than 50%. In contrast, the root length of Br2 (36 ± 4 mm) showed only about 10% reduction with the addition of Al.

Low pH and Al also showed significant effects on root growth of the DH lines. The root length of DH lines ranged from 46 to 405 mm in acid soil, 34 to 116 mm when growing in control solution (pH6.5), 24 to 79 mm in pH4.2 solution and 14 to 41 mm in pH4.2+Al solution (Fig. 3.1). The root lengths of DH lines showed continuous distributions for all the hydroponic treatments. Under acid soil treatment, the root lengths of DH lines were divided into two different groups, indicating major genes controlling the tolerance. Chi-square analysis showed that the segregation did not fit the 1:1 ratio, which is consistent with the significant distortion of markers on the region for Al tolerance gene (Table 3-2).

Table 3-2 Segregation distortion of markers in the region of Al tolerance gene on 4H; * means significance at 0.05, ** means significance at 0.01

Marker	Br2	Hamelin	Missing	$\chi 2$	Significance.
HVM03	86	53	19	7.83	**
Bmag353	103	49	6	19.18	**
Bmac310	84	48	26	9.82	**
Bmac186	101	42	15	24.34	**
Bmag740	88	55	15	7.62	**
Ebmac775	83	54	21	6.14	*
Cit7	104	54	0	15.82	**

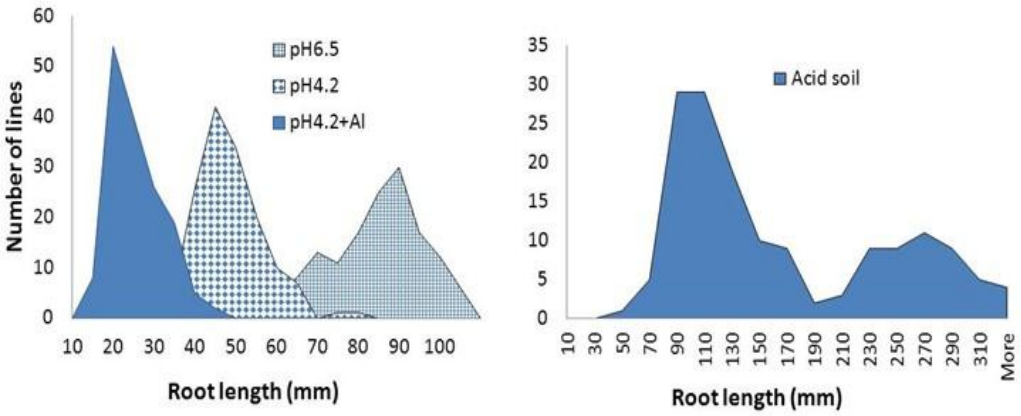

Fig. 3.1 Frequency distributions of root length for Hamelin/Br2 DH population under acid soil, pH6.5, pH4.2, pH4.2+Al treatments

The root lengths of DH lines in hydroponic experiment with pH4.2+Al showed very significant correlation with those in acid soil experiment. The results from hydroponic experiment of pH4.2+Al could explain 62.6% of the phenotypic variation of the root length from the acid soil treatment. The result suggested that the hydroponic experiment with Al was highly consistent with acid soil experiment. However, the root lengths of DH lines growing under pH4.2 showed no correlation with those from acid soil.

A scatter graph was constructed using the phenotypic data from acid soil and pH4.2+Al treatment. As shown in Fig. 3.2, all double haploid lines were clearly classified into two groups. The red scatters signified the sensitive group, while the blue ones represented the tolerant group (52 blue scatters, 102 red scatters and 4 data missing). This indicates that the acid soil/Al tolerance in BR2 is likely controlled by a major gene. As discussed above the biased ratio between tolerant and sensitive lines was caused by molecular marker segregation distortion (Table 3-2).

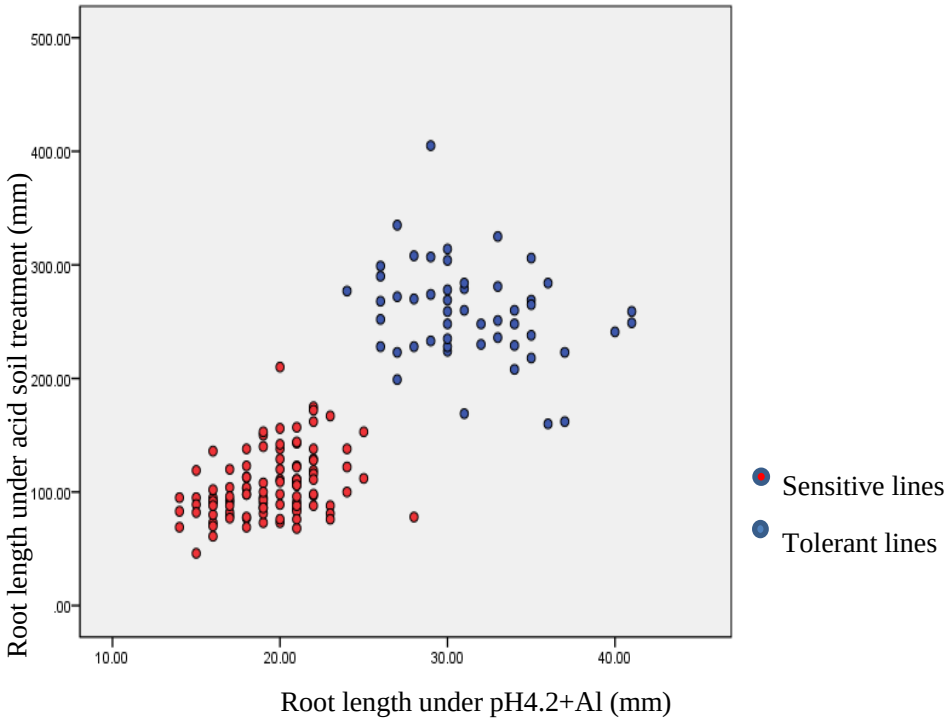

Fig. 3.2 The scatter graph showed Hamelin/Br2 DH lines could be classified into two groups

3.3.2 The linkage map construction and QTL analysis

Thirty-seven lines selected from the double haploid population were genotyped using Diversity Arrays Technology (DArT). In total, 446 commonly-used SSR markers and 388 DArT markers were tested for polymorphism. Of them, 44 SSR markers and 258 DArT markers were distributed on seven barley chromosomes (Fig. 3.3). Preliminary QTL analysis indicated a major QTL for acid soil tolerance on chromosome 4H (data not shown), thus further research was focused on chromosome 4H. Six polymorphic markers in the QTL region were selected to map the whole population and the marker order was similar to previously reported consensus map (see Fig. 3.4) (Varshney et al. 2007). QTL analysis showed that the same QTL controls the root growth in acid soil and pH4.2+Al solutions (Table 3-3). The nearest marker was Bmag353 (Fig. 3.4) and the QTL explained 68.5% of the

phenotype variation of pH42Al, 73.4% of Acidsoil, 78.7% of the average root length of pH4.2+Al and acid soil and 55.5% of RL42Al. When the DH lines were grouped to the tolerant and the sensitive, 88.1% of the variation of the tolerance was explained by Bmag353 (Table 3-3). Based on previous studies, Bmag353 and Bmac310 were the common markers associated with acid soil tolerance gene (Raman et al. 2002b; Wang et al. 2007). Thus, tolerant cultivar Br2 may share the same tolerance gene identified in previous studies.

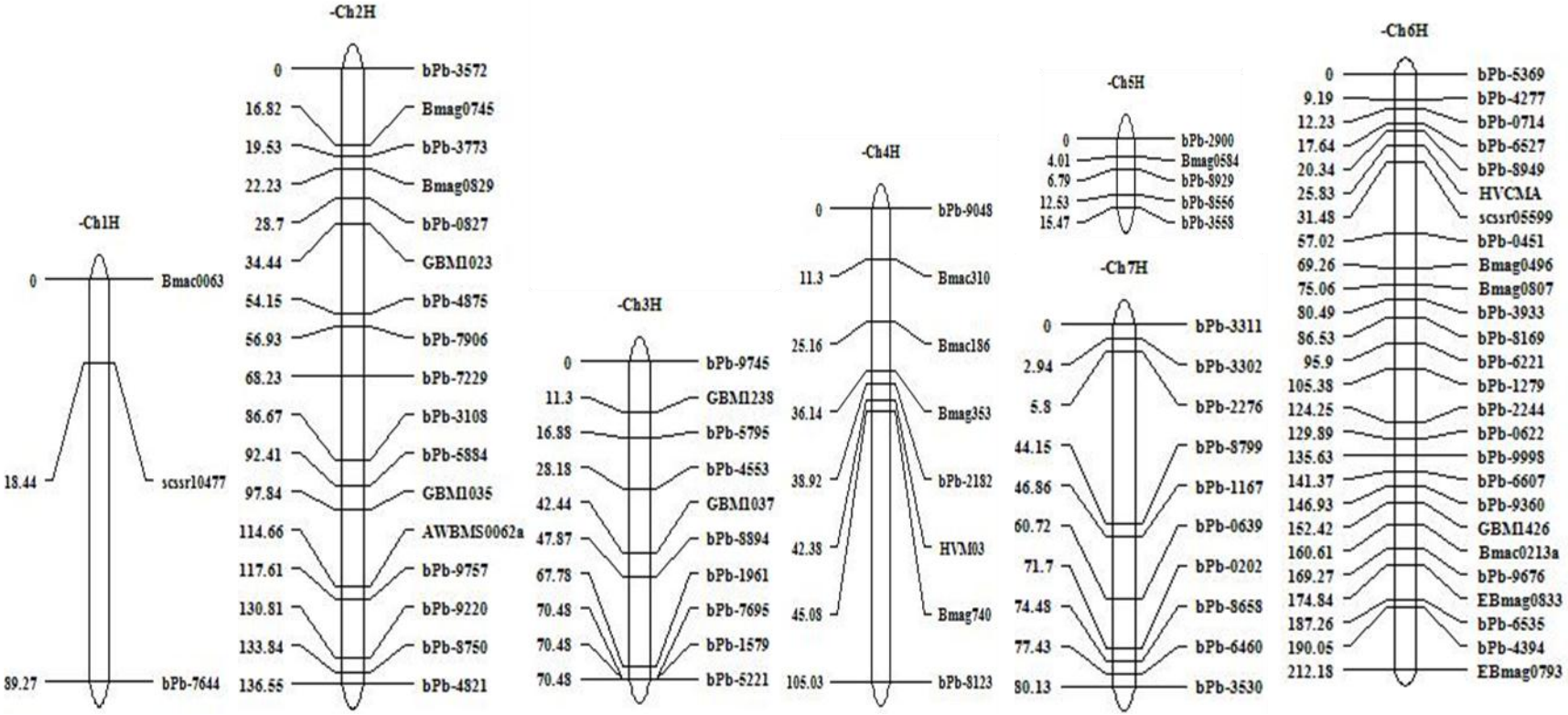

Fig. 3.3 Linkage map of DArT and SSR markers in Hamelin/Br2 DH population

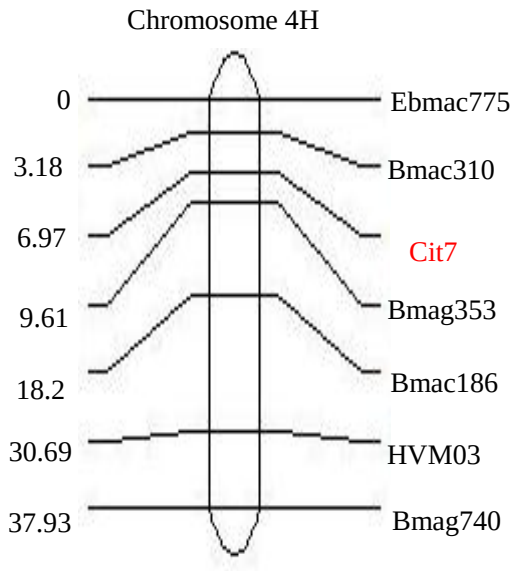

Fig. 3.4 Linkage map of the gene-specific marker Cit7 on chromosome 4H in Hamelin/Br2 DH population

Table 3-3 Phenotype variations of Acidsoil, pH42Al, RL42Al, AvAv and ALT explained by Bmag353 and Cit7 (see Table 3-1 for trait abbreviations)

Trait	QTL location	Before adding cit7		After cit7 added		Cit7 (phenotype variation %)	Bmag353 (phenotype Variation %)
		Lod value	Phenotype Variation	Lod value	Phenotype variation		
pH42Al	4H	35.39	68.48	46.99	75.48	75.7	67.5
Acidsoil	4H	41.64	73.37	56.47	81.98	79	72.4
RL42Al	4H	24.4	55.48	33.26	63.03	63	55.4
AvAv	4H	48.57	78.7	70.49	87.88	87.9	78.1
ALT	4H	-	-	-	-	100	88.1

3.3.3 Gene-specific marker development and association analysis

HvAACT gene contains 14 exons and 13 introns with 12257 bp full length (Fig. 3.5A). Forty-four pairs of primers covering 5342 bp upstream region and a 6362 bp downstream region were designed to amplify the fragments of *HvAACT* gene in Hamelin and Br2 (Chapter 2 Fig. 2.8). The PCR product size of these primers varied from 200 bp to 500 bp. Of them, 16 pairs of these primers were found to be polymorphic between Hamelin and Br2 (see Table 3-4 and Fig. 3.5B). One marker Cit7 (Cit7F: 5-GCAGCCAAGACCTTGAGAAAGC-3 and Cit7R: 5-GCCTGAACTAGCCCGAGAAATG-3) designed from the coding region of *HvAACT1* gene and other 54 polymorphic SSR markers were used to construct the linkage map. The result showed that the gene specific marker Cit7 was located between SSR markers Bmag353 and Bmac310 on chromosome 4H (Fig. 3.4). As it can be seen from Fig. 3.6 and Table 3-3, when the new marker Cit7 was integrated into the linkage map of chromosome 4H, the phenotypic variation explained by the QTL increased for all the traits. For example, 82% of the variation in root length in acid soil can be explained by the QTL compared with 73% explained previously (see Table 3-3). Association analysis between markers and phenotypic data showed that the new marker Cit7 is more precise in explaining the phenotype variation than the other markers under acid soil treatment. Fig. 3.7 shows that Cit7 accounted for 79% of the variation under acid soil treatment, while Bmag353 explained 72% of the phenotypic variation. Similar trends were found for other traits, pH42Al, RL42Al, AvAv and ALT (Table 3-3).

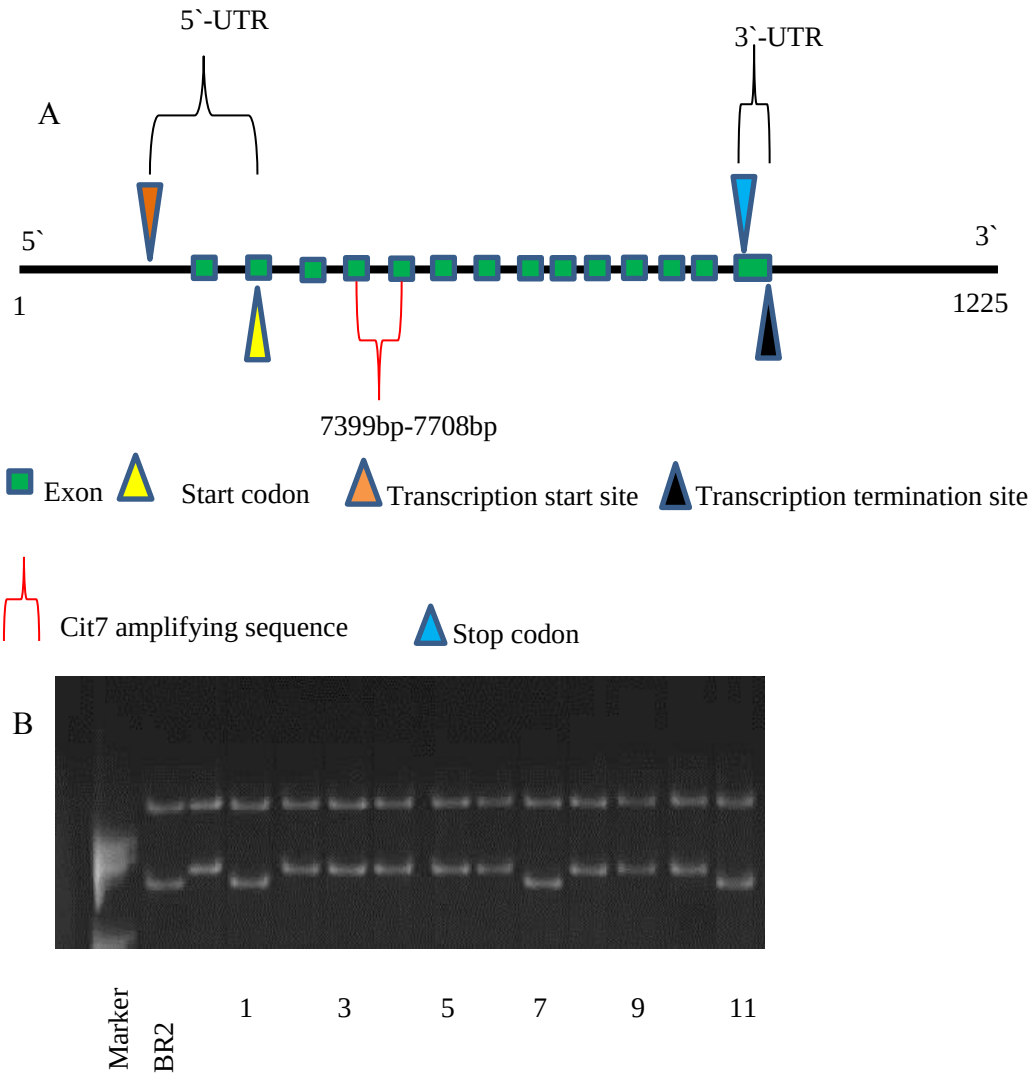

Fig. 3.5 The structure of *HvAACT1* gene and the polymorphism detected by Cit7. A, gene structure of *HvAACT1* gene and the amplifying region of Cit7; B, the polymorphism detected by Cit7 in DH population lane 1 to 11 were Hamelin/Br2 DH lines

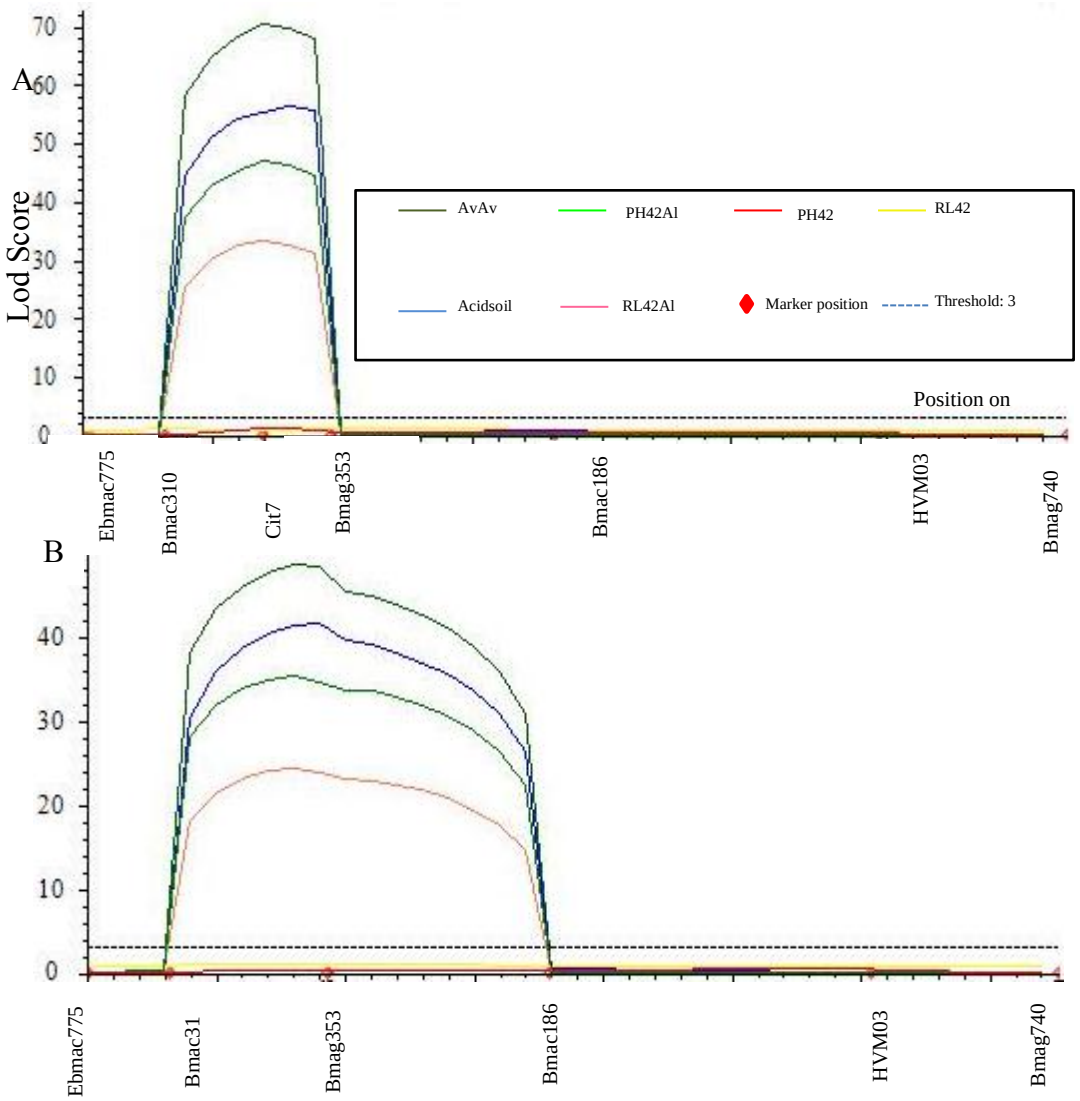

Fig. 3.6 QTL and LOD score for acid soil tolerance on chromosome 4H in Hamelin/Br2 population. A: for commonly-used SSR markers and gene-specific marker Cit7; B: for commonly-used SSR markers

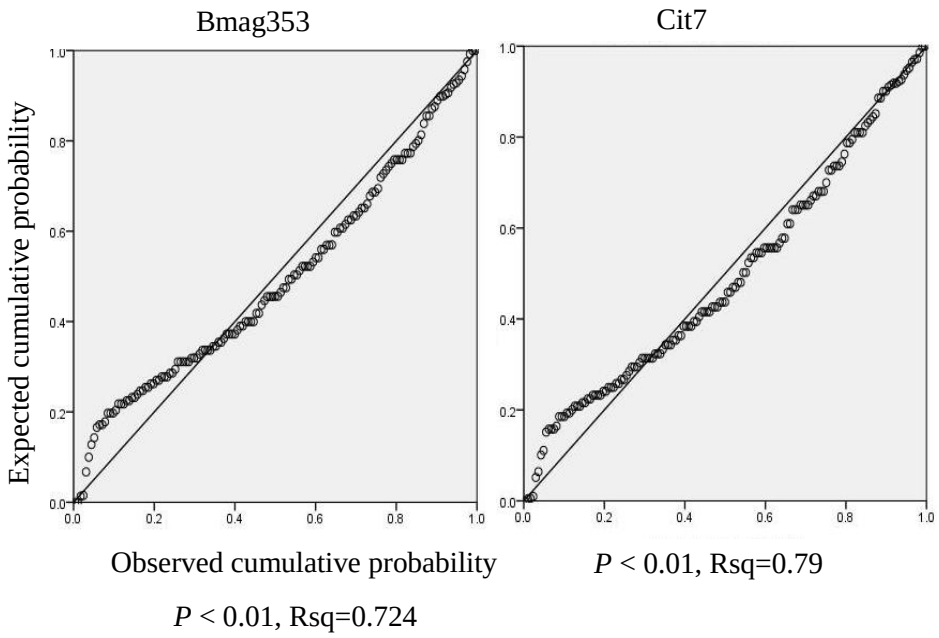

Fig. 3.7 Association analysis of Cit7 and Bmag353 using Hamelin/Br2 DH population under acid soil treatment

Table 3-4 Polymorphic markers derived from *HvAACT1* gene between Hamelin and Br2

Marker name	Product length (bp)	Annealing temperature (^{0}c)	Polymorphic type	Primers
HvMATE-21indel	497	62	Agarose	F: GCTAGGGCTTGAAAACTGTTTG R: GACGAACTGTACGATGATGATGC
D0	352	55	Agarose	F: GCCACGCCTTACAGTAAAGAAC R: CCTAGCTATCTCAAGTTGGCTTAC
Cit7	312	67	SSCP	F: GCAGCCAAGACCTTGAGAAAGC R: GCCTGAACTAGCCCGAGAAATG
Cit14	225	58	SSCP	F: TCGGGTATTGGAGTTAGAAGGG R: CGGGCACATTTGATGCAAGGAT
Cit16	205	55	SSCP	F: CCCGAGTTATGTCATTTTTCCTCTC R: GGGCCTGGTTGGGCCTTAT
Cit6	225	55	SSCP	F:ACCTTCCGTGACATCTGCTCTA R:ATCGGTGAGTCCTGGAATAGTG
U5	491	55	SSCP	F:CACACAACTGGAAAACAACTACC R:GGATAAAACTTCAGTGCGACG
Cit1501	332	55	SSCP	F: GAAGGGGCCTATTGCTTCAC R: CACCCATAAGTTGTGGTTCGG
Cit19	355	67	SSCP	F: TGGTGAAACGGGCATGTCTC R: GAAACCAGGTATATTGCAAGAGC
U12f+U11r	726	55	Agarose	F: TCGTCAATCGCAACTCTCAGA R: CATATCGTTTGTCGTATCACGC
U4	404	55	SSCP	F:CAAGTGTGAAATAGAGAGTCGGTAG R:CGCAAGAACATTTTTGTCACG
D5	453	55	SSCP	F:CGGGTATTGGAGTTAGAAGGG R:GCTATAAAGTCCACGCTATGCAG
D3	448	58	SSCP	F:CTCCTGCGAGGCAGATGAG R:CTCGCTCTCCCTAATGGTGG
D01R	362	60	SSCP	F:GCTCAACCAGACTCAGGTAAGC R:CCAAACAGGGCCTAAGCTTC
D202rr	474	60	SSCP	F:GTCTTCAACAGCATGATTAAGGTC R:CAAACCTAGCACTATTCGGGTG
D4FF	440	58	SSCP	F:CAATCCTTGCATCAAATGTGC R:GGCCCTAAGATAGAAGCACAAG

3.3.4 Sequencing and prediction of the amplifying region function

The sequences of Cit7 amplifying PCR products were aligned using the software Genious 5. The result showed that Cit7 covered partial sequence of *HvAACT1* gene exon 4 (7255-7461bp) and partial sequence of exon 5 (7572-7765bp) (Fig. 3.5). DNA sequencing showed one SNP (T (sensitive)-G (tolerant)) between sensitive and tolerant cultivars (Fig. 3.8).The online translation software expsy-translate tool (available from http://web.expasy.org/translate/) was used for DNA translation under default parameters. The result showed that the SNP caused one single amino acid change (L (172)-V) between the sensitive and the tolerant (Fig. 3.8).

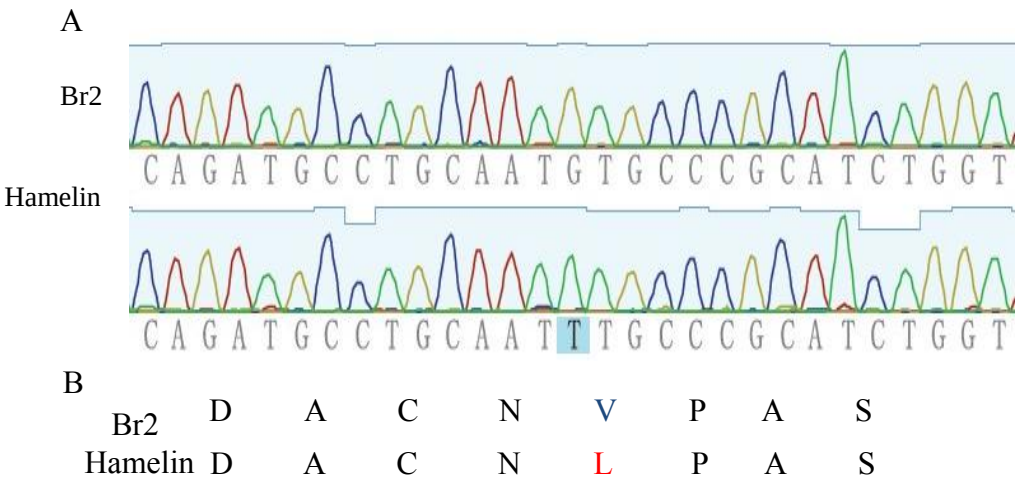

Fig. 3.8 DNA sequencing of Br2 and Hamelin. A: Sequencing showed a common SNP between two parents; B: the DNA translation showed that the SNP caused one amino acid transition between two parents

3.4 Discussion

3.4.1 A single gene controls the tolerance to Al toxicity in the tolerant cultivar Br2

Acid soil limits plant growth through pH toxicity and metallic toxicity, especially Al toxicity (Kochian et al. 2004). The present phenotyping results showed that low pH had a significant impact on root growth and the combination of low pH and Al showed even greater effects on root growth (Fig. 3.1). Both parents were sensible to low pH. However, Br2 showed much better Al tolerance with less reduction in root length after the addition of Al to pH4.2 solution compared to the reduction for Hamelin.

It is still not fully understood whether Al tolerance in plants is a quantitative or qualitative trait (Kochian et al. 2004). In some plants, such as rice (Nguyen et al. 2002; Nguyen et al. 2003; Famoso et al. 2011), maize (Ninamango-Cárdenas et al. 2003) and triticale (Zhang et al. 1999) the tolerance was proved to be quantitative, while in other plants, such as wheat (Sasaki et al. 2004), Pea (Singh and Choudhary 2010) and Chickpea (Singh and Raje 2011) it was inherited monogenetically. Even for different genotypes in same species, the inheritance can be different (Collins et al. 2008). Most studies showed that the tolerance of barley to Al is controlled by a single gene (Wang et al. 2006; Minella and Sorrells 1997; StøLen and Andersen 1978). However, there are still some reports suggested that the Al tolerance of barley could be a quantitative trait. For example, Navakode (2009) reported that the Al tolerance was controlled by three QTLs, which were located on chromosome 2H, 3H and 4H, respectively. Two QTLs were detected chromosome 2H and 4H respectively under 10 μM Al, while one QTL was found on chromosome 3H at 20

μM Al (Navakode et al. 2009). In the present study, the scatter graph (Fig. 3.2) showed only two distinctive groups, indicating that the Al tolerance in tolerant cultivar Br2 was controlled by a single gene. The two groups of the DH lines were compared with the scoring result of Cit7 and the phenotype and genotypes showed a perfect match. Our results supported most of the previous studies that only one gene is responsible for Al tolerance in most accessions (Minella and Sorrells 1997; StøLen and Andersen 1978; Echart et al. 2002).

Segregation distortion is a common phenomenon in barley which skews the frequency of alleles from their Mendelian expectations (Li et al. 2010b). The distortion of the markers in the region of the tolerance gene (Table 3-2) in this DH population caused the biased segregation ratio of the tolerant to the sensitive, which did not fit 1:1 (χ^2 = 16.23). Further studies are needed to confirm whether all the genes/loci reported in different studies are the same.

3.4.2 QTL analysis and marker efficiency

The Al/acid soil toxicity is caused by excessive exposure to soluble toxic metallic element and lack of sufficient essential elements in low pH condition (Foy et al. 1978). The tolerance to acid soil may comprise of low pH tolerance and Al tolerance. In the present study, all the DH lines were severely affected by low pH and no QTL can be detected for root length. The major difference between DH lines was Al tolerance with a major QTL being detected for root length and relative root length of pH4.2+Al treatment. The QTL was located in a same position of that for root growth under acid soil conditions. As most of the current acid soil tolerant varieties are tolerant to Al toxicity but not necessarily to low pH, the searching of germplasm tolerant to low pH may be the key for future breeding programs which

target on acid soil tolerance.

Bmac310 and Bmag353 were the most commonly used SSR markers in barley acid soil study. Both markers were tightly linked with Alp locus and could be effectively used in marker assisted selection (Raman et al. 2003). A candidate gene *HvMATE* was validated on chromosome 4H and more markers such as ABG715 and HvGABP were found to be closely linked with the locus (Wang et al. 2007). The same gene was also validated in another population (Furukawa et al. 2007). Compared with the commonly used SSR markers, Bmac310 and Bmag353, the new marker Cit7 was more precise in explaining the phenotypic variation under acid soil treatment (Table 3-3).

3.4.3 Single nucleotide polymorphism can affect the gene function

Attributed to decades of studies on acid soil/Al tolerance in plants, several genes controlling Al tolerance have been detected, such as *TaALMT* in wheat (Sasaki et al. 2004), *ScAACT1* and *ALMT1* gene cluster in rye (Collins et al. 2008; Silva-Navas et al. 2011), *AtALMT1* in *Arabidopsis* (Kobayashi et al. 2007), *HvAACT1* in barley (Furukawa et al. 2007) and *ZmMATE1* in maize (Maron et al. 2010). However, it is still not clearly known whether gene sequence variations could affect the gene expression. In a previous study, variation in the sequence of upstream of *TaALMT* gene was proved to affect gene expression (Sasaki et al. 2006). A 1Kb insertion in the upstream of the gene sequence was detected in some tolerant Asian accessions and this indel showed promoter activities (Fujii et al. 2012). In contrast, a different copy-number of *ZmMATE1* gene was found to be responsible for phenotypic variation between one Al-tolerant parent and one sensitive parent maize (Maron et al. 2013).

In the present study, one SNP in the coding region of *HvAACT1* was detected between sensitive cultivar and tolerant cultivar and validated by DNA sequencing. DNA translation showed one amino acid change between sensitive cultivar and tolerant cultivar. The SNP marker was further validated in other 56 accessions. These accessions were collected from Australia, New Zealand, Japan, China and European countries and acid soil treatment was applied to test the Al tolerance. The result showed the root length varied greatly from 37mm to 184 mm. Two alleles (same as Br2 and Hamelin, respectively) were detected using Cit7. The regression analysis showed allele one (the same as Hamelin) responded for 11% ($p < 0.05$) of the phenotypic variation and allele two (the same as Br2) responded for 14% of the phenotypic variation ($p < 0.05$) (Fig. 3.9). It is possible that the change of the amino acid can affect gene function (Schaefer and Rost 2012; Choi et al. 2012), which has been confirmed by many studies (Doyle and Amasino 2009; Chono et al. 2003). For example, the protein of a mutant clf-59 contains one Pro-to-Ser amino acid transition in a Cys-rich region. This mutant was reported to elevate levels of trimethylation on lysine 27 of histone H3 (H3K27me3) and repressed FLC (FLOWERING LOCUS C) during vernalization in Arabidopsis (Doyle and Amasino 2009). In barley, the Thr/Ala-233 and Ala/Ser-885 substitutions in limit dextrinase gene were found to be associated with enzyme thermostability (Yang et al. 2009). Although the full sequence of the gene in two parents cultivars have not been obtained and there could be other sequence variations causing phenotype variation, the potential for the single amino acid change to affect the gene function cannot be neglected. More evidence is needed to prove its role in gene expression.

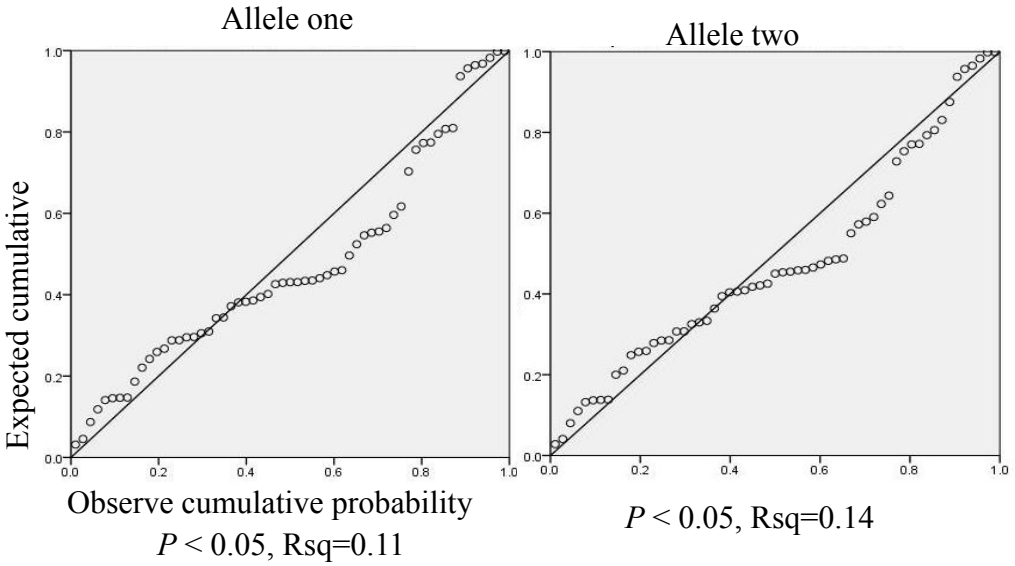

Fig. 3.9 Association analysis of two alleles from Cit7 using 58 accessions under acid soil treatment

In conclusion, the present study identified a new source of acid soil/Al tolerance from a Brazilian cultivar Br2. This variety showed very good tolerance to Al toxicity but sensitive to low pH. A new gene-specific marker Cit7 was developed based on the sequence of the *HvAACT1* gene. The phenotypic variation was more precisely predicted by this new marker compared with other published markers (79% by Cit7 compared with 72% by Bmag353 in acid soil). The gene-specific marker developed in this study will improve the efficiency of molecular assisted selection of new barley varieties with tolerance to acid soil.

3.5 Reference

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Chapter 4 – Allelic variations and association mapping analysis of Al tolerance based on *HvAACT1* gene in barley (*Hordeum vulgare*. L)

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Allelic variations and association mapping analysis of Al tolerance based on *HvAACT1* gene in barley (*Hordeum vulgare*. L). PLoS One (In preparation)

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4.1 Introduction

Most studies have attributed Al tolerance in plant to the extrusion of organic acids (especially citric acid and malic acid) (Bian et al. 2013). Several genes have been reported to be responsible for Al tolerance in different plant species. These include *TaALMT1* in wheat (Sasaki et al. 2004), *ZmMATE1* in maize (Maron et al. 2010), *BnALMT1* and *BnALMT2* in brassica (Ligaba et al. 2006) and *AtALMT1* (Hoekenga et al. 2006) in *Arabidopsis*. However, the modes by which these genes are regulated in different germplasm with multiple levels of Al tolerance still remains poorly understood. More recently, multiple levels of Al tolerance within species was reported to be caused by the sequence variation of responding genes among different germplasm. The repetition of the upstream of *ALMT1* coding region was related to the gene expression and Al tolerance in wheat (Sasaki et al. 2006). In maize, different copies of *ZmMATE1* in tolerant cultivars and sensitive cultivars were reported and the copy numbers were correlated with gene expression (Maron et al. 2013). Even though barley is one of the most sensitive species to Al toxicity among cereals, extensive diversity in Al tolerance exits in barley accessions (Wang et al. 2006). Two genes (*HvAACT1* and *HvALMT*) on chromosome 4H and 2H, respectively, were proved to be responding for citric acid and malic acid exudation, respectively (Furukawa et al. 2007; Gruber et al. 2010). The *HvAACT1* gene was the most well studied gene responding for barley Al tolerance (Bian et al. 2013). In contrast, whether the *HvALMT* is related to barely Al tolerance is still less known (Gruber et al. 2011; Gruber et al. 2010). In the previous chapters, we have showed that Al tolerance in barley is mainly controlled by one single gene, *HvAACT1* on chromosome 4H and no QTLs were detected on other chromosomes. However,

there has been no report focusing on the relationships between allelic variations of *HvAACT1* gene and Al tolerance in barley.

Association mapping has achieved a great success in identifying loci/genes associated with Al tolerance in plant. Two markers on chromosome 3R and three markers on chromosome 4R, 6R, 7R, respectively, were identified to be associated with acid soil tolerance in triticale (Niedziela et al. 2012). In rice, forty-eight regions were found to be associated with Al tolerance (Famoso et al. 2011). Several DArT makers located on chromosome 2H, 7H and 4H were also found to be associated with Al tolerance in Tibetan barleys and it was suggested that novel mechanisms may exist in Tibetan barely (Cai et al. 2013).

There are two commonly used approaches for association mapping in plants. One is genome-wide association studies (GWAS) and the other is candidate gene-based association mapping (CGAM). GWAS is used for searching loci or genes associated with the traits in whole genome without prior information about genomic regions related to interested traits. In contrast, CGAM is aiming at revealing useful sequence variations associated with traits with known information about interested genomic region and has been proved to have more statistical power over GWAS (Amos et al. 2011).

In order to have a better understanding of Al tolerance mechanism, gene-specific markers were developed to cover the whole sequence of *HvAACT1* gene, which were used to test allelic variations in 335 accessions with multiple levels of Al tolerance from different parts of the world. Candidate gene based association mapping was used to identify gene-specific markers associated with acid soil/Al toxicity tolerance.

4.2 Materials and methods

4.2.1 Plant materials and phenotyping

4.2.1.1 Plant material

Three hundred and thirty-five accessions were collected from different parts of the world including Africa, Australia, Brazil, Canada, China, Chile, CYMMIT, Czech Republic, Germany, Japan, Morocco, New Zealand, Pakistan, Portugal, Russia, Spain, Switzerland, Syria, Tibet, UK and the USA. Some accessions were of unknown origin (for the names and origins of accessions, see Appendix Table A-2). 247 accessions were selected to conduct the association mapping analysis.

4.2.1.2 Phenotyping using hydroponic culture

Three hydroponic treatments were used to screen barley for their tolerance to soil acididy and Al: i) control treatment at pH 6.5; ii) acid treatment at pH 4.2 and iii) acid + Al treatment at pH 4.2 with 2 ppm Al concentration.

All three treatments contained the same nutrient solution with the following macronutrients (mM): $CaCl_2 \cdot 2H_2O$, 4.0; $(NH4)_2SO_4$, 0.1; KNO_3, 6.5; $MgCl_2 \cdot 6H_2O$, 2.5; NH4NO3, 0.4 and the following micronutrients (µM): NaH_2PO4, 13; $MnSO_4 \cdot H_2O$, 2; $CuSO_4 \cdot 5H_2O$, 0.3; $ZnSO_4 \cdot 7H_2O$, 0.8; H_3BO_3, 10; $Na_2MoO_4 \cdot 2H_2O$, 0.1 and $FeSO_4 \cdot 7H_2O$, 10. In the acid + Al treatment, Al was included as $AlCl_3 \cdot 6H_2O$ at 74 µM (2 ppm Al). The pH of the solutions was adjusted to either 6.5 or 4.2 using KOH or HCl.

Approximately 50 seeds of each line were placed in a 9 cm Petri-dish with 5 ml DI water. Extra seeds of each line were included (approximately the double amount) to ensure there were enough seeds with similar root lengths for the experiment. The

Petri-dishes were wrapped in Clingfilm in bundles of 10-20 and the seeds were incubated in the dark for 45 hours at 4ºC, then 47-48 hours at 18ºC.

Germinated seeds were placed into allocated positions on grid trays ensuring that roots were kept moist during the process. The seeds were positioned so that roots were facing downwards into the nutrient solution. Seeds with root lengths between 4-7 mm were selected preferentially. The first replication was sown on the first day and the second replication was sown on the second day. Germinated seeds were stored at 4ºC between different sowing times. The grid trays containing germinated seeds were placed into three treatment solutions and grown at 20/15ºC (day/night) in a controlled environment with a 12-hr light per day. All solutions were bubbled gently with air stones and aquarium pumps to aerate the solutions and prevent stagnation. The pH of the solutions was checked daily and adjusted using either KOH or HCl. Dosing meters, which dosed acid or alkali, were also used to maintain pH at desired levels.

The length of the longest root was measured after 7 days. Data were spatially analyzed to take into account any positional effects.

4.2.1.2 Phenotyping with soil bioassay

Natural acid soil was collected from the 10–30 cm layer in Merredin Western Australia. Soil pH was 4.1 with soluble aluminium of 8.1 mg/kg. For the control treatment, lime was added to the same soil to adjust the pH to 6.5. Five seeds of each line were sown in pots containing acid or limed acid soil. The plants were grown for one week after emergence, and then removed from the soil for root length measurements. Root length was used as a parameter for acid soil tolerance. The

experiment was conducted in a glasshouse and each experiment was repeated three times.

4.2.2 Genotypic scanning of accessions

A set of 1662 DArT markers (see Fig. 4.1) and 44 pairs of gene-specific primers or primer combinations was used to screen the accessions. The DArT markers were run using Diversity Arrays Technology (DArT) (http://www.diversityarrays.com) with the barley version 2.0 array. DNA extraction, PCR reaction, the gene-specific marker development, gel electrophoresis and sequencing method of the 44 pairs of primers were reported in Chapter 2.

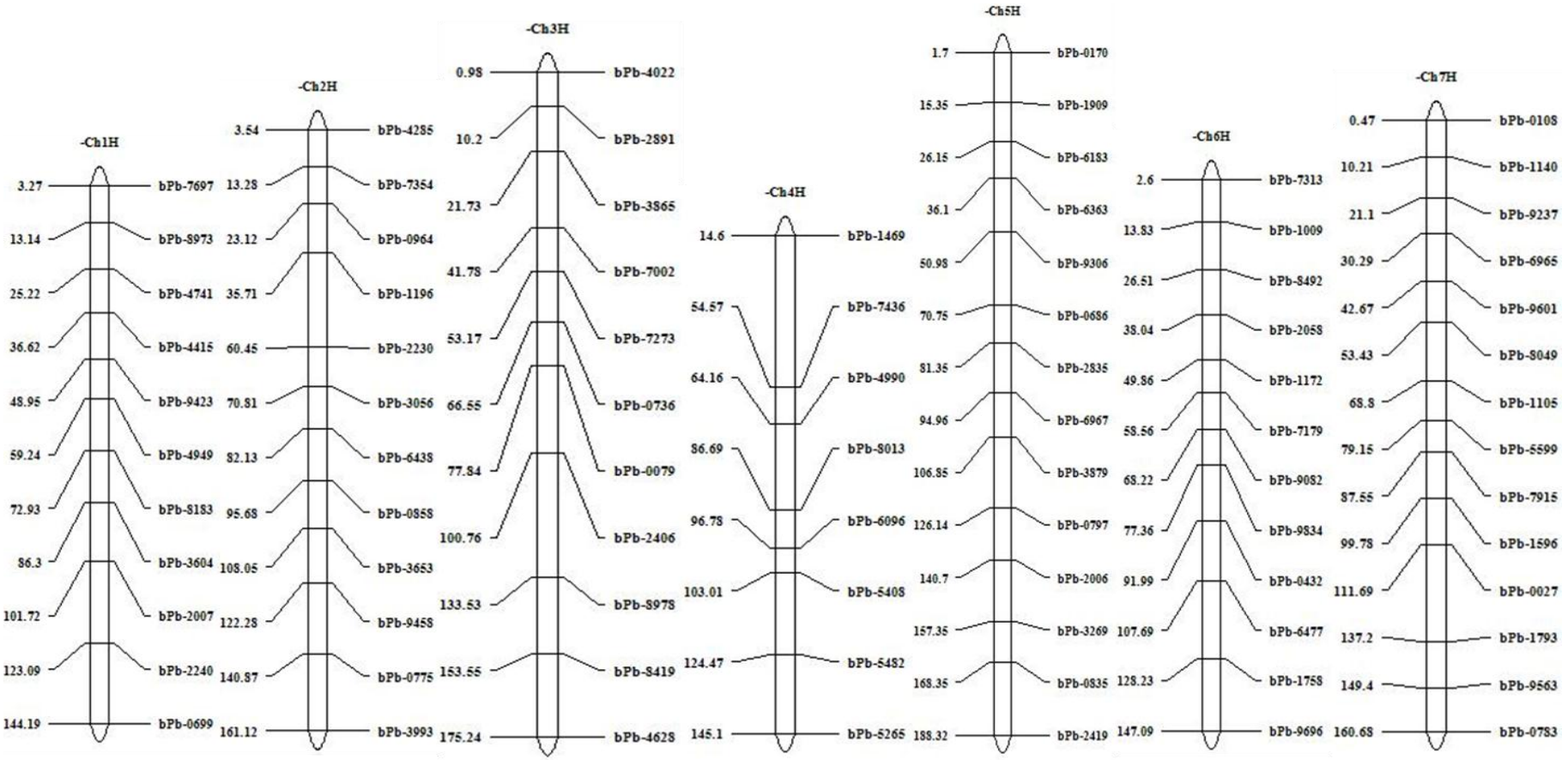

Fig. 4.1 Distribution of DArT markers on seven chromosomes of barley (some markers were deleted due to too much density)

4.2.3 Data analysis

4.2.3.1 The phenotypic data collection and analysis

A total of seventeen traits were collected. Table 4-1 lists the abbreviations and details for these traits (Table 4-1). PASW Statistics v.18 (SPSS Inc., Chicago, USA) was used to perform statistical analysis.

Table 4-1 The abbreviations and details of the seventeen traits

Abbreviation	Meaning
Av42	Average root length under pH4.2 (mm)
Av42Al	Average root length under pH4.2+Al (mm)
Av65	Average root length under pH6.5 (mm)
RGpH	Av42/Av65
RGAl	Av42Al/Av65
Meansoil	Average root length under acid soil (mm)
MeanLime	Average root length under acid soil treated with lime (mm)
Change	Meansoil/MeanLime
Mean2011	Mean root length under acid soil in 2011 (mm)
Mean2012	Mean root length under acid soil in 2012 (mm)
Mean1112	Mean root length under acid soil in 2011 and 2012 (mm)
B20MeanAcid	Average root length in acid soil (mm)
B20MeanLime	Average root length under acid soil treated with lime (mm)
B20perofLimed	B20MeanAcid/B20MeanLime
ASmean07	Average root length under acid soil in 2007 (mm)
ASmean10	Average root length under acid soil in 2010 (mm)
CNTRSmean10	Average root length under control acid soil in 2010 (mm)

4.2.3.2 Genetic diversity analysis

Each polymorphism of the gene-specific primers or primer combinations was scored using two systems. In the first system (Type 1) each band of one specific primer was given a number to differentiate it from the others. In the second system (Type 2), data were assembled in binary format (presence = 1, absence = 0) and each band amplified by one marker was treated as one allele (Gong et al. 2009; Breseghello and Sorrells 2006). Polymorphism information content (PIC) for each primer was calculated to estimate its allelic variation according to the formula: PIC = $1 - \sum P_{ij}^2$, where P_{ij} is the frequency of the i^{th} allele for marker j and the summation extends over n alleles (Botstein et al. 1980). One dendrogram demonstrating the genetic relationships between different accessions was constructed by the unweighted pair group method with arithmetic average (UPGMA) using DARwin 5.0 software based on the dissimilarity matrix calculated according to the simple matching coefficient equation: $d_{ij} = \frac{b+c}{2a+(b+c)}$, where d_{ij} is the dissimilarity between units i and j, a is the number of variables where x_i = presence and x_j = presence, b is the number of variables where x_i = presence and x_j = absence and c is the number of variables where x_i = absence and x_j = presence (Perrier X et al. 2003).

4.2.3.3 Structure analysis

The genetic structure of 247 accessions was constructed by STRUCTURE V2.3.1 software using 1662 DArT markers (Falush et al. 2003; Pritchard et al. 2000). Subpopulation numbers from 2–10 were modeled with a burn-in of 50, 000 cycles, followed by 100, 000 iterations with ten independent runs using an admixture model. Posterior probability Pr (X|K) of each K was generated by the program and lnPr (X|K) was plotted against each value of K. The final result was

uploaded to structure harvester (Earl and Vonholdt 2012). The most probable value of K was detected by an ad hoc quantity (ΔK), which is related to the second order change in the log probability of data with respect to the number of clusters inferred by STRUCTURE (Evanno et al. 2005). For an optimal K with 10 runs, the one with the highest likelihood value of lnPr (X|K) was selected to assign the posterior membership coefficients to each accession.

4.2.3.4 Linkage disequilibrium and association analysis

Linkage disequilibrium (LD) plot and genotype/phenotype associations were studied by means of the mixed linear model (MLM) using TASSEL software version 3.0 (http://www. maizegenetics.net/). The values of squared correlation coefficient (r^2) and the significance of any LD detected between polymorphic sites were evaluated with a Fisher's two-tailed test. Association analysis was based on the polymorphic markers of *HvAACT1* genes and the phenotypic traits under different treatments of 247 accessions from 335 accessions (see Appendix Table A-2).

4.3 Results

4.3.1 Phenotypic variation of different accessions

Different accessions showed a wide range of variations in root length under different treatments (Table 4-2). The control treatments (Av65, MeanLime and CNTRSmean10) showed relatively higher mean values than the other treatments. Under the hydroponic experiments, Av65 had the highest mean root length, followed by Av42 and Av42Al. Similarly, root lengths in lime treated soil were much higher than those in acid soil. Root lengths of different accessions under soil

treatments showed broader variations than the root length under hydroponic treatments with Meansoil varying from 9 mm to 197 mm while Av42Al varying from 12 mm to 43.7 mm.

Pearson correlation was conducted between the 17 traits (Table 4-3). Most traits were significantly correlated with each other. Traits derived from the hydroponic experiment showed good correlation with the traits derived from experiment with plants growing in the acid soil. For example, Av42Al and RGAl significantly correlated with Meansoil negatively. This indicated that hydroponic experiment could well imitate acid soil experiment. The result was consistent with Chapter 3.Traits derived from the control experiment also showed correlation with the Al tolerant traits. For example, Av42Al , RGpH and RGAl were negatively correlated with Av65 respectively. Meansoil and Change showed significantly correlated with Meanlime. This may indicate that there are some commonly shared genes are responsible for the root length both under control experiment and Al tolerance experiment.

Table 4-2 Descriptive statistical analysis of the seventeen traits

Trait	Minimum	Maximum	Mean	Standard Deviation
Av42	23.65	101.80	53.79	19.54
Av42Al	12.00	43.70	23.3	6.86
Av65	54.00	121.00	88.42	16.15
RGpH	30.20	136.40	63	26.88
RGAl	12.60	57.30	27.42	10.67
Meansoil	9.00	197.33	71.15	39.17
MeanLime	88.00	327.50	193.78	47.5
Change	3.23	109.20	38.09	20.92
Mean2011	2.50	8.00	4.95	1.5
Mean2012	2.00	12.00	6.32	1.81
Mean1112	1.25	9.75	5.34	1.6
B20MeanAcid	37.38	183.75	80.12	31.4
B20MeanLime	91.25	181.57	146.82	18.29
B20perofLimed	23.47	106.17	54.6	19.16
ASmean07	0.75	6.58	2.37	1.24
ASmean10	1.00	8.33	3.32	1.2
CNTRSmean10	3.25	12.75	8.63	1.82

Table 4-3 Pearson correlation analysis of the phenotypic traits, T1-T17 stand for Av42, Av42Al, Av65, RGpH, RGAl, Meansoil, Meanlime, Change, Mean2011, Mean2012, Mean1112, B20Meanacid, B20Meanlime, B20peroflime, Asmean07, Asmean10 and CNTRSmean10, * and ** stand for significance at 0.05 and 0.01 level respectively

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17
T1	1																
T2	-	1															
T3	-	-0.229**	1														
T4	0.86**	0.188*	-0.449**	1													
T5	-	0.857**	-0.661**	0.378**	1												
T6	-	-0.201*	-0.209*	-	-0.241*	1											
T7	-0.303**	-	0.24*	-0.371**	-0.245*	0.192*	1										
T8	-	-	-	-	-	0.863**	-0.278**	1									
T9	-	-	-	-	-	-	-	-	1								
T10	-	-	-	-	-	-	-	-	0.555**	1							
T11	-	-	-	-	-	-	-	-	0.826**	0.902**	1						
T12	-	-	-	-	-	-	-	-	0.659**	0.314*	0.519**	1					
T13	-	-	-	-	-	-	-	-	0.320*	0.338*	0.334**	1					
T14	-	-	-	-	-	-	-	-	0.592**	-	0.390**	0.933**	-	1			
T15	-	-	-	-	-	-	-	-	0.513**	0.580**	0.556**	0.401**	-	0.338*	1		
T16	-	-	-	-	-	-	-	-	0.473**	0.380**	0.448**	0.436**	0.301*	0.360**	0.516**	1	
T17	-	-	-	-	-	-	-	-	0.506**	0.396**	0.516**	0.517**	0.411**	0.404**	0.413**	0.641**	1

4.3.2 Genotype screening

1662 DArT markers distributed on 7 chromosomes were found to be polymorphic among different accessions. Fig. 4.1 shows the positions of only parts of DArT markers on 7 chromosomes. The PIC value of the DArT markers varied from 0.031 to 0.5 with an average value of 0.358. Of the forty-eight gene-specific markers, thirty-seven markers showed polymorphic among these 335 accessions. After removing redundant ones (markers covering the same polymorphic region and none-polymorphic markers), twenty-seven markers were reserved for future analysis. Of them, 6 pairs of primers were located in the upstream region of the gene, while 14 and 7 were located in the coding and downstream region of the gene, respectively. Polymorphisms of 25 markers were identified on 12% SSCP gel (Fig. 4.2), while the other 2 were detected on 2% agarose. Eighty-two alleles were revealed by these twenty-seven markers with PIC ranging from 0.502 (Cit3) to 0.90 (D4FF) (Table 4-4).

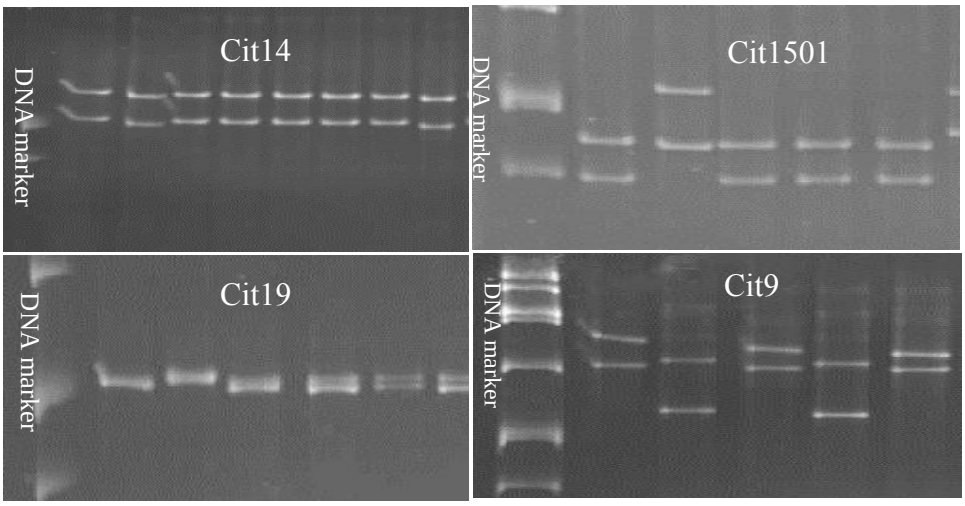

Fig. 4.2 Polymorphisms of four markers of Cit14, Cit1501, Cit19 and Cit9 in different barley accessions

Table 4-4 Polymorphic gene-specific marker sequences, allele numbers and PIC values

Marker name	Allele number	PIC value	Polymorphic type	Annealing temperature	Marker sequence
Cit6	2	0.51	SSCP	55	F:ACCTTCCGTGACATCTGCTCTA
					R:ATCGGTGAGTCCTGGAATAGTG
Cit18	2	0.50	SSCP	55	R:TATTCTTACCAGTAGCATACAACGG
					F: ACATCTGCTCTAATCGTTGGCTC
Cit7	2	0.75	SSCP	67	F: GCAGCCAAGACCTTGAGAAAGC
					R: GCCTGAACTAGCCCGAGAAATG
Cit3	2	0.50	SSCP	67	F: CTCACGACCATCCAACCAACAT
					R: CCAAATCAAGCGACCGATACTG
Cit14	2	0.75	SSCP	58	F: TCGGGTATTGGAGTTAGAAGGG
					R: CGGGCACATTTGATGCAAGGAT
Cit16	4	0.82	SSCP	55	F: CCCGAGTTATGTCATTTTTCCTCTC
					R: GGGCCTGGTTGGGCCTTAT
Cit1001	2	0.65	SSCP	55	F: CTCAATGAATTGAAACTCTGGG
					R: CGGTGACGTTCTGTGTCACTC
Cit9	2	0.62	SSCP	60	F: TCTTGATATGTCGGCTCGTCCA
					R: GGGAATATGATTTGGCGGTCTG
Cit1501	4	0.82	SSCP	55	F: GAAGGGGCCTATTGCTTCAC
					R: CACCCATAAGTTGTGGTTCGG
Cit19	2	0.75	SSCP	67	F: TGGTGAAACGGGCATGTCTC
					R: GAAACCAGGTATATTGCAAGAGC
Cit501	2	0.50	SSCP	55	R: CTTCTAGGTATTTGCTAATGATGGC
					F: TGCTGACAATAGCGCCCAC
Cit12	2	0.50	SSCP	55	F: AAACTTGTCCCGAGACTGAAAT
					R: CCGAAGAAATGGAGGTACTGAT
Cit0	2	0.51	SSCP	62	R: CCATACACAGCGGATGCTTG
					F: CTTTTGCTTCCGCACATAGC
Cit1	2	0.51	SSCP	58	F: CCGGTATAAGTAGGGGTGAGCT
					R: GCACTCGTAAGACGTACAGTATG
U12F+U11R	3	0.75	Agarose	55	F: TCGTCAATCGCAACTCTCAGA
					R: CATATCGTTTGTCGTATCACGC
U5	2	0.71	SSCP	55	F:CACACAACTGGAAAACAACTACC
					R:GGATAAAACTTCAGTGCGACG
U4	4	0.84	SSCP	55	F:CAAGTGTGAAATAGAGAGTCGGTAG
					R:CGCAAGAACATTTTTGTCACG
U2	3	0.67	SSCP	58	R:CAGTCAGTCTGTCAGATGTGGCT
					F:GGTCGATCACGCAACGTAAC
U6	5	0.83	SSCP	55	R: GGGTAGTTGTTTTCCAGTTGTG
					F:CCGTCAAAGATGCATATAACC
HvMATE-21indel	3	0.78	SSCP	62	F: GCTAGGGCTTGAAAACTGTTTG
					R: GACGAACTGTACGATGATGATGC
D0	5	0.85	SSCP	55	F: GCCACGCCTTACAGTAAAGAAC
					R: CCTAGCTATCTCAAGTTGGCTTAC

Table 4-4 (continued)

Marker name	Allele number	PIC value	Polymorphic type	Annealing temperature	Marker sequence
D5	4	0.83	SSCP	55	F:CGGGTATTGGAGTTAGAAGGG
					R:GCTATAAAGTCCACGCTATGCAG
D3	2	0.72	SSCP	58	F:CTCCTGCGAGGCAGATGAG
					R:CTCGCTCTCCCTAATGGTGG
D01R	2	0.67	SSCP	60	F:GCTCAACCAGACTCAGGTAAGC
					R:CCAAACAGGGCCTAAGCTTC
D202RR	6	0.87	SSCP	60	F:GTCTTCAACAGCATGATTAAGGTC
					R:CAAACCTAGCACTATTCGGGTG
D4FF	8	0.90	SSCP	60	F:CAATCCTTGCATCAAATGTGC
					R:GGCCCTAAGATAGAAGCACAAG
INT0RR+INT2F	2	0.51	Agarose	62	F:CTTCATTTCAACCAAGCACTCC
					R:GCTTTTGGTCGAACAAAGTATCG

4.3 3 Cluster and structure analysis

Cluster analysis was conducted using the 82 alleles from gene-specific markers by DARwin software (Perrier X. and Jacquemoud-Collet 2006). 334 accessions were classified into three groups (Pavlovicky was not in the dendrogram due to too much data missing) (Fig. 4.3). Principal coordinates analysis based on the dissimilarity matrix was also constructed among 334 individuals to test the effectiveness of the alleles in differentiating accessions using DARwin 5.0 software. Results showed that 334 accessions were divided into 3 groups by cluster analysis and were well separated by the principal coordinates analysis (Fig. 4.4). The first two axes accounted for 51.06% and 12.73% of the total variation respectively.

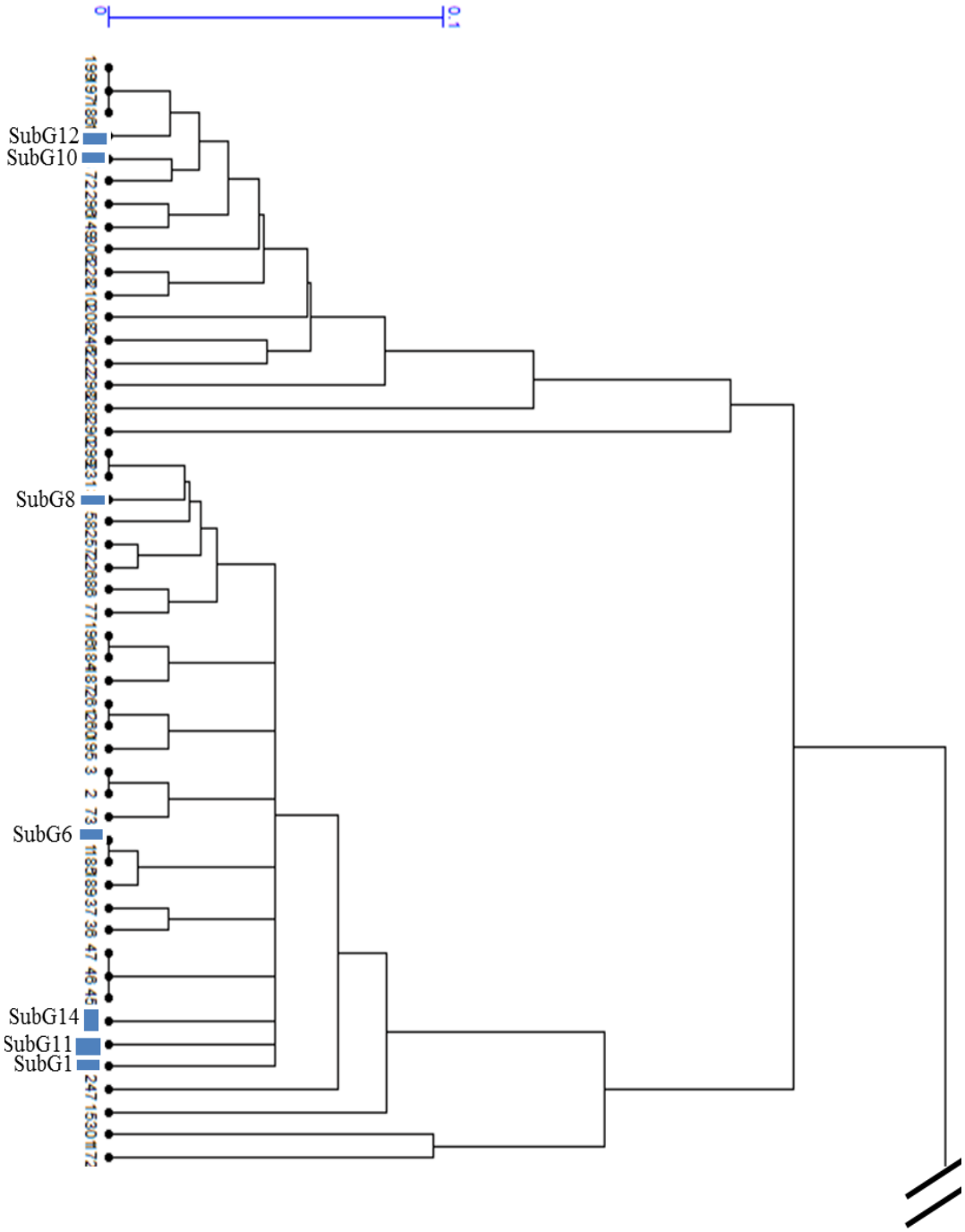

Fig. 4.3 UPGMA dendrogram describing genetic relationships among 334 barely accessions (accession names and subgroups were listed in Appendix Table A-3) – first part

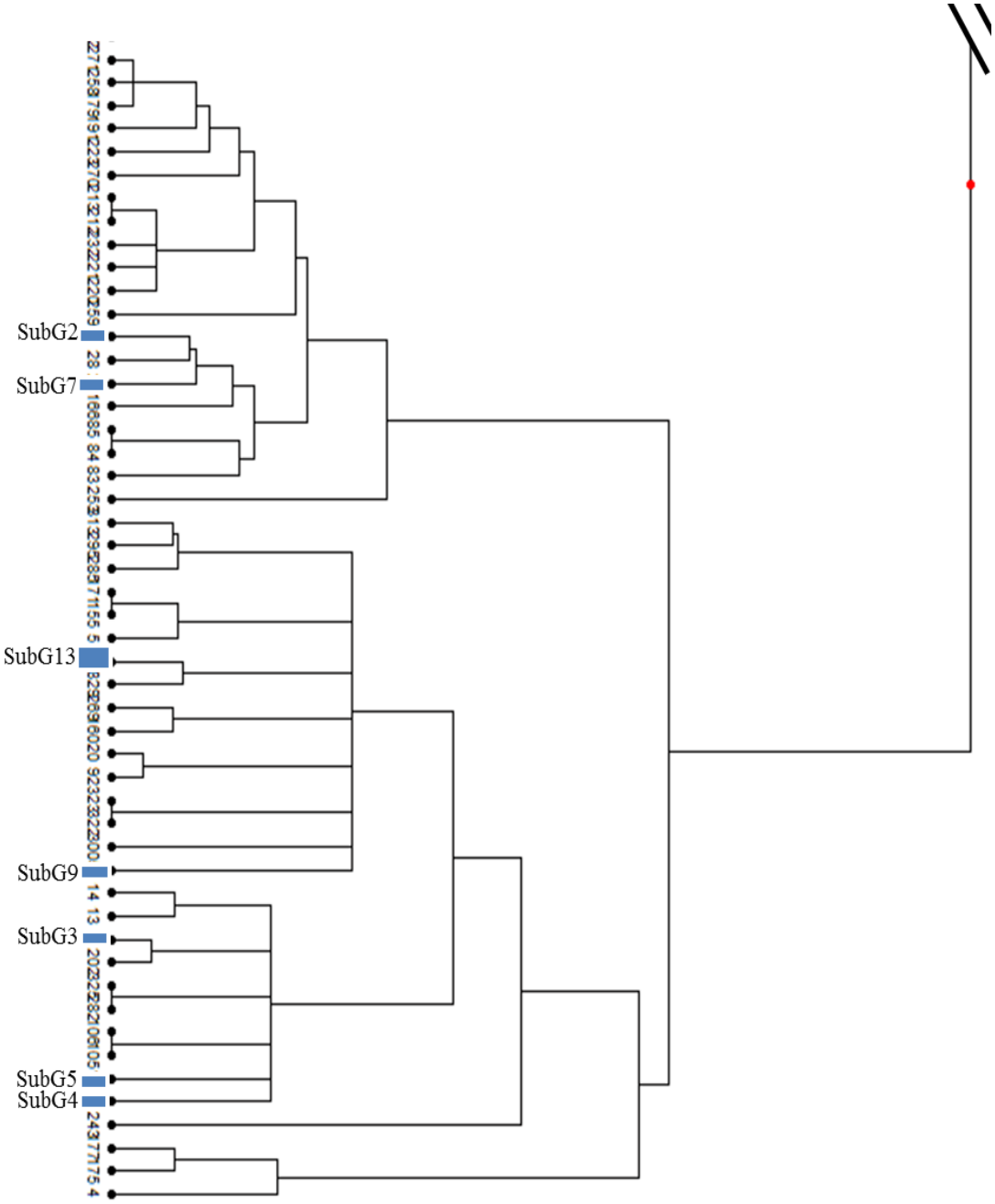

Fig. 4.3 UPGMA dendrogram describing genetic relationships among 334 barely accessions (accession names and subgroups were listed in Appendix Table A-3) – second part

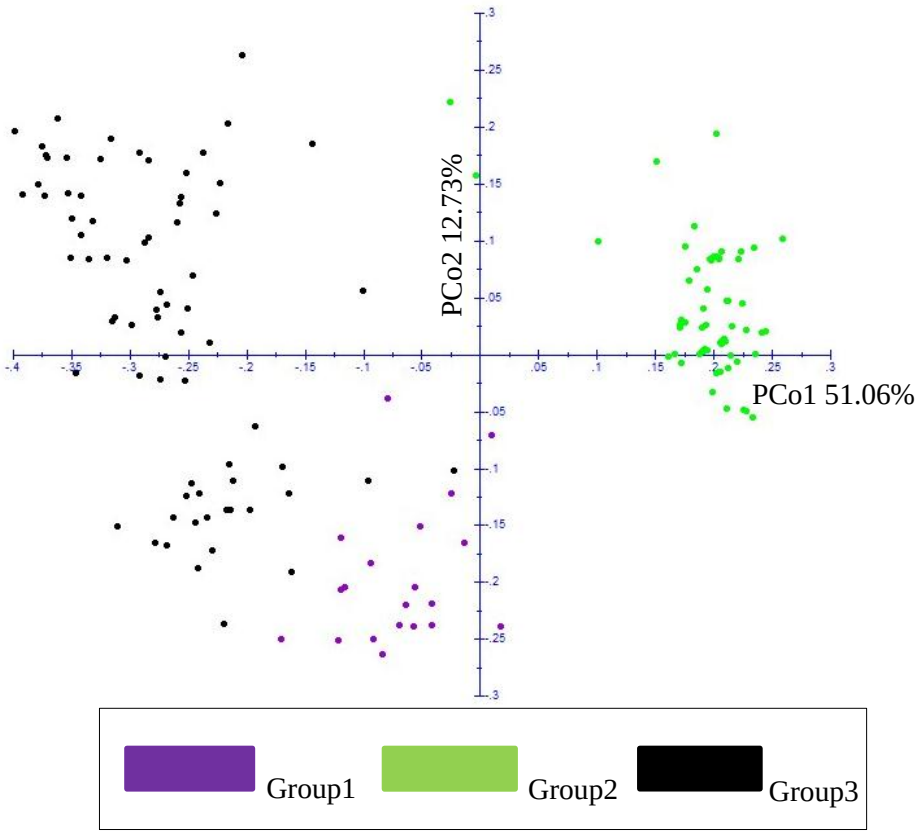

Fig. 4.4 Principal coordinates analysis of 334 accessions. Scatter plots represent the grouping of accessions according to the first component versus the second one. The three colours represent the different groups identified by cluster analysis

Structure analysis on 247 accessions using 1662 DArT markers showed that the log likelihood revealed by STRUCTURE increased gradually from K=2 to K= 9 and no obvious optimal K was found (Fig. 4.5). The ad hoc quantity (ΔK) was used (Evanno et al. 2005) to overcome the difficulty in choosing the real K value. A relatively high value of ΔK for 247 accessions was found for K = 6. At K = 6, a relatively high value of ΔK and high probability of accessions assigned to one specific cluster (Fig. 4.6).

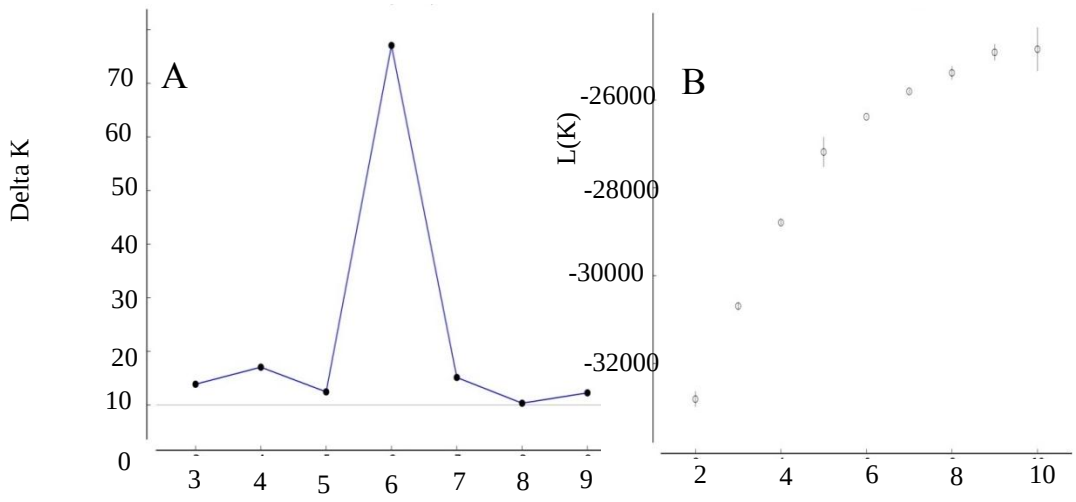

Fig. 4.5 Estimation of the most probable number of populations (k), based on 10 independent runs and k ranging from 2 to 10. (A) The rates of change in log likelihood for models with K = 2 to 10, showing the optimal K = 6; (B) The log likelihood profiles for models with K = 2 to 10

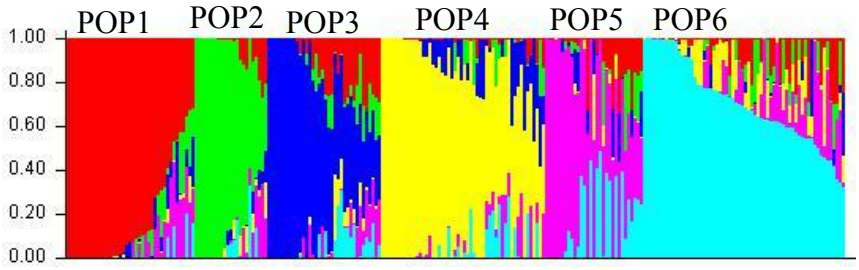

Fig. 4.6 Population structure of 247 barley accessions based on genetic diversity detected by 1662 DArT markers. Each of the 247 barley accessions is denoted as a vertical line, with the six subgroups represented by different colors

4.3.4 Candidate gene based association mapping analysis

The accessions with more than two alleles when amplified using one marker were treated as missing data. The correlation squared (r^2) was used. Mixed Linear Model (MLM) implemented in TASSEL software (http://www. maizegenetics.net/)

was applied using genotypic data derived from each polymorphic gene specific primer or primers` combination and 17 phenotypic traits (Bradbury et al. 2007; Yu et al. 2006). Sixteen markers were related to twelve traits at $p < 0.01$ level and the R square varied from 0.28% to 14.7%. Four markers, Cit14, Cit1501, Cit19 and Cit9 in the coding region were associated with six traits, CNTRSmean10, Average42Al, Average42, Average65, Change and Meansoil, respectively (Table 4-5); D0, D01R, D1rr, D202RR, D4FF and D5 in the downstream region were associated with nine traits including MeansLime, Average65, Change, Meansoil, B20MeanAcid, Average42, Mean1112, Mean2012 and RGpH, respectively. Markers in the upstream region, INT0RR+INT2F, U12F+U11R, U2, U4 and U6, were related to Average42Al, B20MeanAcid, Change, CNTRSmean10, Meansoil and RGAL.

Table 4-5 Significance (*P* value) and phenotypic variation (R^2) explained by individual markers associated with Al tolerance traits ($P < 0.01$)

Trait	Marker	*P* value	R^2 (%)
Average42	D4FF	0.0013	3.96
	Cit19	0.002	1.64
Average42Al	INT0RR+INT2F	2.55E-04	2.56
	Cit1501	0.0045	2.01
Average65	HvMATE-21indel	7.73E-07	4.23
	D01R	0.0022	2.01
	Cit9	0.0028	1.45
B20MeanAcid	HvMATE-21indel	0.0041	9.77
	U12F+U11R	0.0077	7.37
Change	D4FF	2.78E-06	4.6
	INT0RR+INT2F	5.07E-05	1.96
	U4	1.20E-04	2.72
	U6	3.20E-04	2.49
	U12F+U11R	6.18E-04	1.42
	Cit9	0.0013	1.27
	D202RR	0.003	2.37
	D01R	0.0076	1.13
CNTRSmean10	U6	0.0018	10.46
	U4	0.0034	9.69
	Cit14	0.004	5.51
Mean1112	D4FF	0.0063	14.34
Mean2012	D4FF	0.0096	14.7
MeansLime	D4FF	4.34E-14	5.28
	D202RR	1.90E-05	2.42
	D0	3.77E-04	1.45
	D5	0.007	0.98
Meansoil	D4FF	2.67E-06	4.23
	INT0RR+INT2F	8.91E-05	1.67
	D202RR	1.99E-04	0.28
	U4	2.28E-04	2.34
	U12F+U11R	4.99E-04	1.34
	U2	9.54E-04	1.06
	Cit9	0.002	1.06
	D01R	0.002	1.17
	U6	0.0036	1.72
RGAL	INT0RR+INT2F	2.06E-04	2.66
RGpH	D4FF	6.26E-06	6.32

4.3.5 Haplotype analysis

Haplotype block analysis of markers was conducted by sliding window LD with 5 marker sets as the LD window size using TASSEL v3.0 software. Closely linked markers which were at $r^2 > 0.1$ and $P < 0.001$ level were considered as a haplotype block. A total of six haplotype blocks were detected (Fig. 4.7). The numbers of markers constituting haplotype blocks varied from 2 to 6. Markers associated with phenotypic traits were within or around these haplotypes. Based on allelic combinations for different markers observed in each haplotype block, the numbers of haplotypes in each haplotype block were 19 (haplotype block 1), 3 (haplotype block 2), 6 (haplotype block 3), 4 (haplotype block 4), 30 (haplotype block 5) and 20 (haplotype block 6) respectively. Haplotype block 1, 5 and 6 were clustered into 3, 3 and 4 groups, respectively, and each group was considered as a haplotype due to too many allelic combinations. Associated traits were grouped based on either allelic combinations or cluster groups. An ANOVA test at $\alpha = 0.05$ level was conducted among traits grouped by different haplotypes. The haplotypes of traits with significant difference were listed in Table 4-6. Haplotype block 4, 5 and 6 could well separate four traits including Change, Meansoil, CNTRSmean10 (control) and B20MeanAcid, respectively.

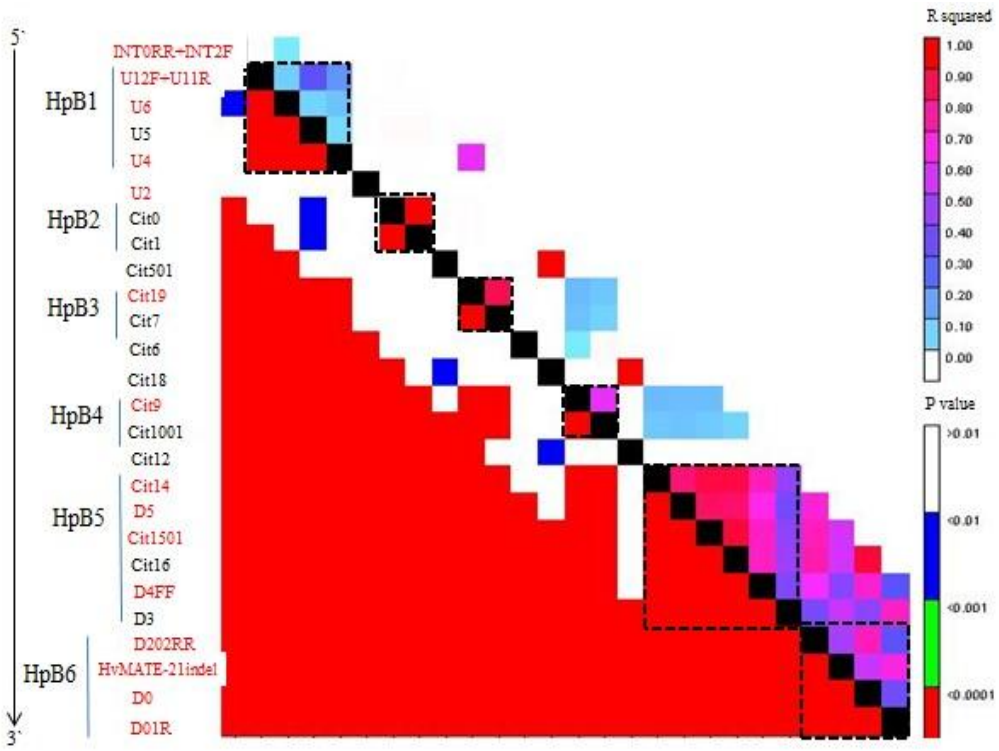

Fig. 4.7 Haplotype blocks (HapB) defined by sliding window LD of 5 marker sets at the level of $r^2 > 0.1$ and $P < 0.001$. Red markers are those significantly associated with phenotypic traits

Table 4-6 Haplotypes and the grouped significant traits, capital and small letters show significance at $P < 0.01$ and $P < 0.05$ when comparing haplotype for each trait. F: Frequency; HapB: Haplotype block; Hp: Haplotype, capital and small letters show significance at $P < 0.01$ and $P < 0.05$ when comparing haplotype for each trait

HapB	Marker number	Hp	F(%)	Change		Meansoil		CNTRSmean10		B20MeanAcid	
				Mean±S.E	Range	Mean±S.E	Range	Mean±S.E	Range	Mean±S.E	Range
HapB1	4	HB1-1	91.02	-	-	-	-	-	-	-	-
		HB1-2	4.08	-	-	-	-	-	-	-	-
		HB1-3	4.9	-	-	-	-	-	-	-	-
HapB2	2	HB2-1	1.22	-	-	-	-	-	-	-	-
		HB2-2	98.37	-	-	-	-	-	-	-	-
		HB2-3	0.41	-	-	-	-	-	-	-	-
HapB3	2	HB3-1	0.84	-	-	-	-	-	-	-	-
		HB3-2	46.41	-	-	-	-	-	-	-	-
		HB3-3	52.74	-	-	-	-	-	-	-	-
HapB4	2	HB4-1	14.46	60.83±4.6B	16.89-109.2	122.76±9.12B	32-197.33	-	-	-	-
		HB4-2	5.37	35.3±3.5A	18.88-54.15	65.93±5.65A	40.58-105.25	-	-	-	-
		HB4-3	80.16	32.6±1.59A	3.23-106.76	58.84±2.34A	9-187.33	-	-	-	-
HapB5	6	HB5-1	45.75	28.3±1.21A	3.23-53.25	51.63±1.71A	9-80.83	7.81±0.3A	3.25-10.25	-	-
		HB5-2	42.51	48.98±2.86B	16.89-109.2	91.42±5.31B	30.17-197.33	9.86±0.36B	6.75-12.75	-	-
		HB5-3	11.74	27.42±2.45A	16.38-52.94	53.74±2.93A	39.17-78.83	8.54±0.36A	7.5-10.5	-	-
HapB6	4	HB6-1	46.75	28.28±1.19A	3.23-53.25	51.57±1.69A	9-80.83	-	-	72.65±4.39A	45.6-157.5
		HB6-2	11.79	27.42±2.45A	16.38-52.94	53.74±2.93A	39.17-78.83	-	-	65.19±6.79A	37.38-93.25
		HB6-3	15.45	55.94±4.33B	16.89-109.2	114.78±9.2B	32-197.33	-	-	108.45±12.3B	52.43-157
		HB6-4	26.07	44.4±3.74C	17.86-106.76	76.82±5.5C	30.17-187.33	-	-	106.29±14.69B	60.25-183.75

Haplotype block 4 can well separate Change and Meansoil into different groups. The three haplotypes in Haplotype block 4 separated two traits into one tolerant group and two sensitive groups respectively. All the accessions in tolerant group obtaining Cit1001A1 and Cit9 A1 alleles, while the other two sensitive groups obtaining the allele combination of Cit1001A1 and Cit9 A2 and allele combination Cit1001A2 and Cit9 A2, respectively. A similar trend could also be observed in Haplotype block 5 and Haplotype block 6. In Haplotype block 5, the leading accessions (42 in 105) in tolerant group obtaining allele combinations of Cit14 A2, D5 A3, Cit1501 A2, Cit16 A1, D4FFA3 and D3A2 while the leading accessions in other two sensitive groups (23 in 29 and 98 in 113) obtaining the allele combination of Cit14 A2, D5A6, Cit1501A3, Cit16 A3, D4FF A2 and D3A2and allele combination Cit14 A1, D5A2, Cit150 A1, Cit16A2, D4FFA4 and D3A1, respectively. In Haplotype block 6, The leading accessions (17 in 38) in tolerant group obtaining allele combinations of D01R A2, D0A1, HvMATE-21indelA2 and D202RRA3 while the leading accessions in medium tolerant groups (in the case of B20MeanAcid, this group has no difference with the tolerant group) (32 in 64) obtaining the allele combination of D01RA2, D0A1, HvMATE-21indelA2 and D202RRA2. The leading accessions in two sensitive groups (22 in 29 and 94 in 115) obtaining the allele combination of D01R A1, D0A2, HvMATE-21indelA3and D202RRA7 and allele combination of D01R A1, D0A3, HvMATE-21indel A3 and D202RR A4, respectively.

4.3 6 Sequencing analysis

The polymorphic regions covered by gene-specific markers varied from more than 1 kb indel (U6) to 1 SNP (Cit19). The coding region sequences covered by

polymorphic markers, Cit19, Cit9, Cit1501 and Cit14, were compared by Genious 5 software. The sequences of polymorphic regions were translated to amino acid sequences using online software expsy-translate tool (available from http://web.expasy.org/translate/) under default parameters. The result showed that the polymorphisms included some SNPs and indels: Cit9 - a G (Hamelin) -A (Svanhals) synonymous SNP; Cit19 - one amino acid change (L (Hamelin) -V (Svanhals)); Cit14 - a 2-base pair indel and one SNP in the 3` UTR and a SNP in the downstream of 3` UTR; Cit1501 - one 2-base pair indel in 3` UTR (the same indel region as Cit14), one 1 - base pair indel in 3` UTR, two 1 - base pair indel in the downstream of 3` UTR and 6 SNPs in the downstream of 3` UTR (Fig. 4.8).

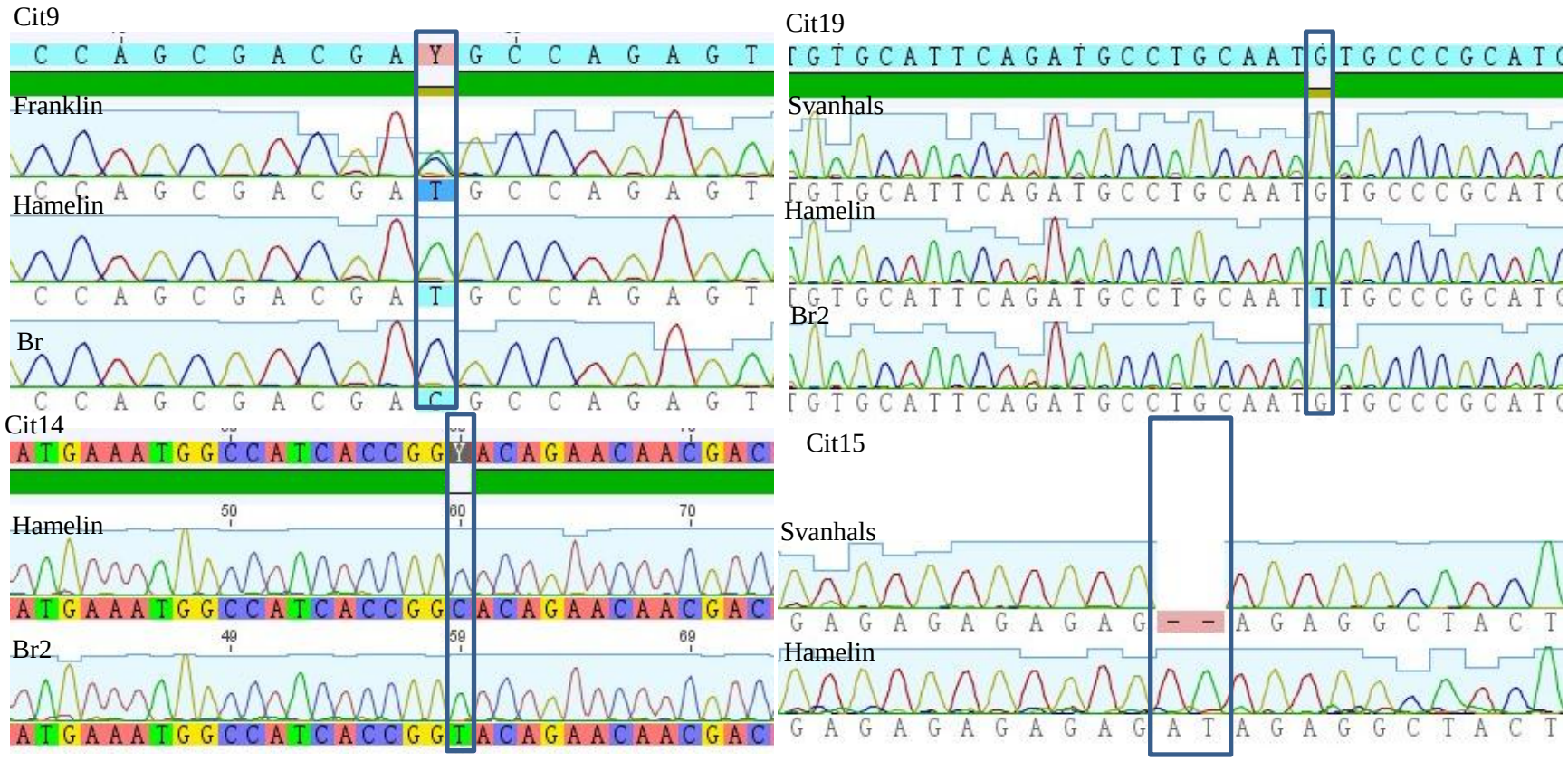

Fig. 4.8 Sequence analyses of four polymorphic markers, Cit14, Cit1501, Cit19 and Cit9 in different barley accessions (the first two markers locate in non-coding region, while the latters locate in coding region).

4.4 Discussion

4.4.1 Variation of phenotypic traits among accessions

Accessions grew normally and the root length varied significantly under controlled hydroponic conditions. When pH was decreased to 4.2, the root growth was severely reduced, and when Al was added to pH 4.2 solutions, the root growth range was more severely inhibited than single pH factor. The result is in consistent with Chapter three and many other previous studies that both the low pH and Al can cause toxic effect and dual factors can cause much severer effect than single one (Kinraide 1998; Delhaize and Ryan 1995). It was reported that the Al^{3+} and H^{+} interacted with each other and the low pH can increase the solubility of Aluminum, which may lead to a severer toxic effect (Ohyama et al. 2013). Some accessions showed little root growth reduction under pH 4.2 treatment while all the accessions showed significantly decreased root growth under pH 4.2+ Al treatments. This may cause by differences among germplasm to low pH and Al toxicity. For example, *Holcus lanatus* L. and *Betula pendula* Roth races collected from different sits differed in pH tolerance and Al tolerance. These authors concluded that the races collected from acid organic soil were tolerant to low pH, while the races from acid mineral soils were tolerant to Al but not necessarily tolerant to low pH (Kidd and Proctor 2001).

4.4.2 Gene-specific markers can be used for genetic diversity study

Molecular markers were commonly used tools to characterize and evaluate genetic diversity within or between populations or groups of individuals. Different types of markers have been successfully used in revealing genetic variation in

barley (Matus and Hayes 2002; Wang et al. 2010; Davila et al. 1999). PCR-based gene-specific markers have been also reported to be used in genetic diversity analysis in some plants. Genetic diversity analysis of *Lablab purpureus* L. using 97 pairs of sequence-specific markers showed that the gene-specific markers provided useful information to compare legume genomics and could benefit legume breeding (Venkatesha et al. 2007). This study was the first to use gene-specific markers developed from *HvAACT1* gene to estimate the genetic diversity in Al tolerance of barley. These markers showed a broad range of polymorphisms and higher PIC value than these DArT markers. They could be used in detecting genetic diversity and genotype identification of barley accessions in the future.

4.4.3 Association mapping revealed gene-specific markers associated with Al tolerance traits and pH tolerance traits

Association mapping has been widely used in plant genetic studies (Abdurakhmonov and Abdukarimov 2008). They were reported to have several advantages over genetic mapping including no pedigrees or crosses are required, higher resolution than linkage mapping, haplotype reflection of recombination over very large numbers of generation (Aranzana et al. 2005; Cai et al. 2013). Association mapping from this experiment revealed more polymorphic regions associated with Al tolerance traits compared with QTL mapping in previous chapters and the haplotypes detected could well differentiate Al tolerance related traits. Although there were several Al tolerance studies based on association mapping (Korir et al. 2013; Niedziela et al. 2012; Famoso et al. 2011; Cai et al. 2013), these researches were mainly focused on whole genomic association mapping and there were a few reports on candidate gene based association mapping

on Al tolerance study. This study was the first to use association mapping based on *HvAACT1* gene to detect gene-specific markers associated with Al tolerance in barley. The study revealed that gene-specific markers related to Al tolerance were located in the upstream, coding and downstream regions. Besides the 14 gene-specific markers associated with Al tolerance traits, there were also two markers, Cit19 and D4FF related with low pH tolerance traits, Average42 and RGpH. In the previous study, the gene sequence variation affected phenotypes. Upstream repeats in *TaALMT1* (Sasaki et al. 2006), 1-kb insertion in *HvAACT1* gene (Fujii et al. 2012), miniature inverted repeat transposable elements (MITEs) in the upstream of the *SbMATE* gene (Magalhaes et al. 2007) were all reported to locate in the upstream region. There was no report on if *HvAACT1* gene was related with low pH tolerance. This study was the first to report on two markers developed from *HvAACT1* gene were responsible for low pH tolerance in barley. However, more evidence is needed to prove the role of these polymorphic regions detected in this study.

4.4.4 Sequence variations have potential to affect Al tolerance

Sequencing analysis showed the polymorphic regions varied from more than 1 Kb indel to 1 SNP. Amino acid changes between accessions were detected. As it has been discussed in Chapter 3, it is likely that one amino acid change could result in phenotypic variation between accessions. The indels detected in the upstream of the coding region is likely to have promoter activity as these gene sequence variations in upstream region have been reported in wheat, barley and sorghum (Magalhaes et al. 2007; Fujii et al. 2012; Sasaki et al. 2006).These indels and SNPs in 3` UTR were also likely to have transcriptional control on the gene expression

(de Moor et al. 2005; Kuersten and Goodwin 2003; Merritt et al. 2008). However, more evidence is needed to prove the role of these polymorphic on RNA level.

In conclusion, the present study identified several polymorphic regions associated with Al tolerance in barley using association mapping based on *HvAACT1* gene sequence. The polymorphic regions are located in the upstream, coding and downstream regions. However, more evidence is needed to validate their role in gene expression and acid soil tolerance. These gene-specific markers can be used in genetic diversity study in barley and the associated gene-specific markers can be used for efficient marker-assisted selection of superior alleles in Al tolerance barley breeding programs.

4.5 References

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Chapter 5

Genetic variance of malate transporter gene in barley (*Hordeum vulgare* L.)

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Chapter 6 –General discussion and conclusions

6.1 Introduction

Barley is one of the most important cereals in the world which serves as food, animal feed and malting material to human beings (Bartlett et al. 2008). It is particularly noted for its wide distribution and multiple tolerances to abiotic stress (Jana and Pietrzak 1988; Leff et al. 2004). However, barley is arguably the most acid soil-sensitive among all cereal crops, which makes acid soils to be one of the major factors limiting barley production (Ryan and Delhaize 2010). At the same time, a notable difference in tolerance to acid soil exits among barley varieties. Two main mechanisms are believed to control plant tolerance to Al toxicity (Kochian et al. 2004). The most studied is the external mechanism mediated by excudation of weak organic acids (Delhaize et al. 2007). Several QTLs located on different chromosomes were identified in barley for acid soil tolerance (Navakode et al. 2009; Minella and Sorrells 1997; Raman et al. 2002), and two genes were reported to be responsible for the organic acid exudation. *HvAACT1*, the most studied gene responding for Al tolerance on chromosome 4H, encodes a citrate transporter on cell membrane (Furukawa et al. 2007). *HvALMT*, the function of which was reported to be more likely as anion homeostasis and osmotic adjustment but not Al detoxification, encodes a malate transporter on chromosome 2H (Gruber et al. 2010; Gruber et al. 2011). This study investigated the Al tolerance mechanism of barley using molecular approaches.

6.2 Major finding of this thesis

6.2.1 Genetics of Al tolerance in barley

The genetics of Al tolerance in barley was investigated using two DH populations derived from crosses of Hamelin and two new germplasm, Br2 and Svanhals, respectively. Both acid soil and hydroponic treatments were used to validate the phenotypic variation. All the progenies can be classified into two groups, the tolerant and the sensitive, but the ratios of segregations did not fit 1:1. QTL analysis using SSR and DArT markers showed that there was only one major QTL on chromosome 4H flanked by Bmag353 and Bmag310 ,which are commonly used markers linked with *HvMATE* gene (Wang et al. 2007). The result suggested that the two novel germplasm, Br2 and Svanhals, may have the same gene for Al tolerance as the previous reported (Chapter 2 and Chapter 3) (Wang et al. 2007).

Most of the research attributed the Al tolerance in barley to be single gene-inherited (Minella and Sorrells 1997; StøLen and Andersen 1978; Reid et al. 1971; Echart et al. 2002), although some other studies reported other Al tolerance genes or QTLs on other chromosomes (3H, 5H and 2H) (Navakode et al. 2009; Navakode et al. 2010). The present study was the first to validate the genetics of Al tolerance in the two new tolerant barley germplasm populations. The result was in consistence with the other previous studies that single gene is responding for Al tolerance in barley (Wang et al. 2007; Bian et al. 2013a).

6.2.2 Development of gene-specific markers for Al tolerance in barley

Molecular markers can be effectively used for the marker-assisted selection in breeding programs. Of various markers, gene-specific markers are much preferred

for their directly tracing of interesting genes (Bian et al. 2013b). In some species as wheat, gene-specific markers have been developed based on the polymorphic region of tolerant genes and used in Al tolerance selection (Raman et al. 2008). In the present study, gene-specific markers were developed to cover the whole sequence of *HvAACT1* gene. It was shown that when the gene-specific markers were incorporated into QTL analysis, the phenotypic variation explained by the QTL was much more increased. The developed gene-specific markers could not only facilitate barley acid soil study but also offer better tools for marker-assisted selection for acid soil tolerance in barley. Moreover, the revealed polymorphic regions located on different part of the gene sequence. In a previous study (Fujii et al. 2012), the 1 kb indel in the promoter region was reported to have promoter activity and result in the differences between the tolerant accession from some Asian countries and the sensitive accessions. However, when the INT0rr + INT2f was developed and used to screen the parents, it could not differentiate them while the two markers reported in Chapter 2 and Chapter 3 could well separate the parents.

6.2.3 Gene-specific markers associated with Al tolerance based on *HvAACT1*

Although the gene-specific markers had been developed (Chapter 2, 3, 4 and 5) and some of the gene-specific markers have been validated in double haploid populations (Chapter 2 and Chapter 3), it remained unclear of whether the polymorphic regions covered by these markers are related with Al tolerance. The present study (Chapter 4) used 335 accessions collected from different part of the world to validate their relationships with Al tolerance. In total, 27 gene-specific markers developed from *HvAACT1* gene were significantly associated with the Al tolerant traits. These markers showed well separation of the 335 accessions and a

higher PIC value than these commonly used DArT markers.

6.2.4 Malate transporter gene is related to Al tolerance in barley

In many plants, both malic acid and citric acid transporter genes were responsible for Al tolerance (Sasaki et al. 2004; Hoekenga et al. 2006; Ryan et al. 2009; Liu et al. 2009; Silva-Navas et al. 2011; Collins et al. 2008). However, there was no direct evidence until now for the relationship between malate transporter gene and Al tolerance in barley. In the present study (Chapter 5), the sequence variation of *HvALMT* was investigated among 345 barely accessions. The result showed ten polymorphic regions located in the upstream region and coding region were significantly associated with the phenotypic variation under Al treatments. Ten gene specific markers developed from *HvALMT* gene were significantly associated with the Al tolerance (Chapter 5). These markers showed well separation of the 335 accessions.

6.3 Future work for acid soil tolerance in barley

This study investigated the Al tolerance mechanism in barley using QTL, marker development, association mapping and sequencing techniques under both hydroponic experiment and acid soil experiment. However, there are still some unknown parts which leave several opportunities for further research as a result of this study which are listed below.

6.3.1 New germplasm for gene-specific marker development based on *HvALMT* gene

The present study (Chapter 5) validated that markers developed from *HvALMT* gene were associated with Al tolerance, indicating that *HvALMT* gene also plays

some role for the Al tolerance in some germplasm. The new identified accessions derived populations could be developed to validate the relationship between the sequence variation and Al tolerance using QTL analysis.

6.3.2 Regulation of the gene behavior

Although the gene-specific markers have been developed and validated to be associated with the phenotypic variation, it remains unknown of how the sequence variations affect the phenotypic variation. In some species which show close relationship between phenotyping and responding gene sequence variation (Sasaki et al. 2006; Fujii et al. 2012; Maron et al. 2013), the polymorphic regions were validated at RNA level and were found to affect the gene expression. It is possible that these polymorphic regions covered by these gene-specific markers either developed from *HvAACT1* or *HvALMT* gene could affect the gene expression at RNA level. Future research can be conducted to investigate the relationships between the associated regions and gene expression.

There are some suggestions that amino acid change can cause phenotypic variation. It is likely that these sequence variation of *HvAACT1* or *HvALMT* gene which leads to amino acid change could result in the phenotypic difference. Future research can be conducted to investigate the relationships between the amino acid changes and protein activity. STOP1 was reported to be an essential gene in *Arabidopsis* Al tolerance and the STOP1 mutant caused a lack of AtALMT1 induction under Al treatment in *Arabidopsis* (Luchi et al. 2007). NtSTOP1 regulating Al tolerance gene was also validated in Tobacco (Ohyama et al. 2013). Further research can focus on looking for the regulatory gene of the Al tolerance gene in barley.

6.3.3 Novel mechanism for Al tolerance in barley

It has been validated in many plants that multiple mechanisms coexist in one species. For example, malate exudation, citrate exudation and other mechanisms coexist in rice (Xia et al. 2010; Yamaji et al. 2009) wheat (Sasaki et al. 2004; Ryan et al. 2009), *Arabidopsis* (Hoekenga et al. 2006; Liu et al. 2009; Larsen et al. 2005) and rye (Collins et al. 2008; Silva-Navas et al. 2011). Although the present study demonstrated that *HvAACT1* gene is the major gene responsible gene for Al tolerance in barley and *HvALMT* may play some role in Al tolerance, contribution of other mechanisms cannot be completely ruled out. For example, some other loci besides chromosome 4H and 2H had been reported to be responsible for Al tolerance (Navakode et al. 2009; Navakode et al. 2010). In some accessions from Tibet, makers on 2H and 7H were found to be associated with Al tolerance (Cai et al. 2013). Future research can be on whether the genes located on other chromosomes are the same genes as *HvAACT1* and *HvALMT* or whether other mechanisms rather than organic acid exudation may also exit for Al tolerance in barley.

In conclusion, the present study investigated the Al tolerance mechanism in barley using QTL mapping, marker development, association mapping and sequencing techniques. The result showed that the *HvAACT1*is the major gene for Al tolerance in barley. The *HvALMT* also plays a role in Al tolerance in some accessions. The gene-specific markers developed from both *HvAACT1* and *HvALMT* genes could be used in Al tolerance study and offer efficient tools for marker-assisted selection.

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Appendix

Table A-1 The names and origins of fifty-six accessions used to validate the gene-specific marker

Accession names	Origion	Accession names	Origion	Accession names	Origion
Svanhals	CYMMIT	Spanish Landrace-316	Spain	Macquarie	Australia
Cavmen	Australia	Spanish Landrace-333c	Spain	TF026	Australia
Hindmarsh	Australia	Portuguese Landrace	Portugal	YF374	China
Buloke	Australia	MAR-86-E1138	Unknown	Tx9425	China
Baudin	Australia	Spanish Landrace-336d	Spain	Unicorn	Japan
Cevada de 6 Ordens	Portugal	Spanish Landrace-338c	Spain	Xiaojiang	China
Cevada Preta	Portugal	Vlamingh	Australia	Yan90260	China
Spanish Landrace-352	Spain	HOR13461	Spain	YUQS	China
Spanish Landrace-355	Spain	Cevada de 2 Ordens	Portugal	YWHKSL	China
Noire Maroc	Morocco	Spanish Landrace-349	Spain	Yiwu Erleng	China
Precoce du Maroc	Morocco	Spanish Landrace-349b	Spain	Kinu nijo 6	Japan
Baudin	Australia	93-3143	Unknown	YYXT	China
Boa Fe	Portugal	Lixi 143	China	Zhepi 2	China
Barlis	Morocco	ZUG293	China	YPSLDM	China
Keka	Spain	ZUG403	China	Macquarie	Australia
Rosa	Unknown	YU6472	China	Macquarie	Australia
Gairdner	Australia	Brindabella	Australia		
Moroccan Landrace	Morocco	Numar	America		
Spanish-309d	Spain	Dash	New Zealand		
HOR12517	Spain	Dayton	Australia		

Table A-2 Accession names, origins and the 27 polymorphic markers` gel scoring data, the accessions in bold and italic were used in association mapping analysis, - means missing data

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
Stirling	Australia	1	2	2	2	1	2	1	2	4
Svanhals	CYMMIT	1	2	2	2	1	2	1	2	4
XZ76	China	1	2	2	2	1	2	1	2	4
XZ54/GG1	China	1	2	1	2	2	1	1	2	3
00098	Australia	1	2	1	2	2	1	1	2	2
04048	Australia	1	2	1	2	2	1	1	1	3
04049	Australia	1	2	1	2	2	1	1	1	3
04050	Australia	1	2	1	2	2	1	1	1	3
04051	Australia	1	2	1	2	2	1	2	2	2
04444	Australia	1	2	1	2	2	1	2	2	2
04543	Switzerland	1	2	1	2	2	1	2	2	2
04544	Switzerland	1	2	1	2	2	1	2	2	2
04545	Switzerland	1	2	1	2	2	1	1	2	2
04546	Switzerland	1	2	1	2	2	1	1	2	2
04547	Switzerland	1	2	2	2	1	2	1	2	4
04548	Switzerland	1	2	1	2	2	1	1	2	2
04549	Switzerland	1	2	1	2	2	1	2	2	2
04550	Switzerland	1	2	1	2	2	1	2	2	2
04551	Switzerland	1	2	1	2	2	1	2	2	2
04812	Pakistan	1	2	1	2	2	1	2	2	2
08475	Chile	1	2	1	2	2	1	1	1	3
08484	Brazil	1	2	1	2	2	1	1	1	3
08578	Brazil	1	2	1	2	2	1	1	1	3
08579	Brazil	1	2	1	2	2	1	1	1	3
08580	Brazil	1	2	1	2	2	1	1	1	3
08581	Brazil	1	2	1	2	2	1	1	1	3
08582	Chile	1	2	1	2	2	1	1	1	3
08583	Chile	1	2	1	2	2	1	2	1	3
08584	Chile	1	2	1	2	2	1	2	2	2
08585	Chile	1	2	1	2	2	1	1	1	3
08586	Chile	1	2	1	2	2	1	1	1	3

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
Stirling	Australia	1	1	2	2	2	1	2	2	4
Svanhals	CYMMIT	1	1	2	2	2	1	2	1	4
XZ76	China	1	1	2	2	2	1	2	1	4
XZ54/GG1	China	2	2	2	2	1	2	2	2	12
00098	Australia	2	2	2	2	2	1	2	1	3
04048	Australia	2	2	2	2	2	1	1	2	1
04049	Australia	2	2	2	2	2	1	1	2	1
04050	Australia	2	2	2	2	2	1	1	2	1
04051	Australia	2	2	2	2	2	1	1	1	3
04444	Australia	2	2	2	2	2	1	1	1	3
04543	Switzerland	2	2	2	2	2	1	1	1	3
04544	Switzerland	2	2	2	2	2	1	1	1	3
04545	Switzerland	2	2	2	2	2	1	1	2	3
04546	Switzerland	2	2	2	2	2	1	1	2	3
04547	Switzerland	1	1	2	2	2	1	2	1	4
04548	Switzerland	2	2	2	2	2	1	1	2	11
04549	Switzerland	2	2	2	2	2	1	1	2	3
04550	Switzerland	2	2	2	2	2	1	1	2	3
04551	Switzerland	2	2	2	2	2	1	1	2	3
04812	Pakistan	2	2	2	2	2	1	2	1	3
08475	Chile	2	2	2	2	2	1	1	1	1
08484	Brazil	2	2	2	2	2	1	1	1	1
08578	Brazil	2	2	2	2	2	1	1	1	1
08579	Brazil	2	2	2	2	2	1	1	2	1
08580	Brazil	2	2	2	2	2	1	1	2	1
08581	Brazil	2	2	2	2	2	1	1	2	1
08582	Chile	2	2	2	2	2	1	1	1	1
08583	Chile	2	2	2	2	2	1	1	2	1
08584	Chile	2	2	2	2	2	1	1	2	3
08585	Chile	2	2	2	2	2	1	1	2	1
08586	Chile	2	2	2	2	2	1	1	2	1

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
Stirling	Australia	2	-	3	-	3	2	1	1	1
Svanhals	CYMMIT	2	1	3	3	3	2	1	1	1
XZ76	China	2	1	3	3	3	2	1	1	1
XZ54/GG1	China	2	1	3	2	1	3	-	-	1
00098	Australia	6	1	5	1	1	3	1	-	1
04048	Australia	7	2	3	2	1	1	-	-	1
04049	Australia	7	1	3	2	1	1	2	1	1
04050	Australia	7	-	3	2	1	1	2	-	1
04051	Australia	7	1	3	1	1	3	2	-	1
04444	Australia	7	1	3	1	1	3	2	1	1
04543	Switzerland	7	1	3	1	1	3	2	-	1
04544	Switzerland	7	1	3	1	1	3	2	-	1
04545	Switzerland	7	1	3	1	1	1	1	-	1
04546	Switzerland	7	1	3	1	1	1	2	-	1
04547	Switzerland	1	1	3	3	1	1	1	1	1
04548	Switzerland	7	1	3	1	1	3	2	2	1
04549	Switzerland	7	1	3	1	1	3	2	-	1
04550	Switzerland	7	1	3	1	1	3	2	-	1
04551	Switzerland	7	1	3	1	1	3	2	-	1
04812	Pakistan	6	1	1	1	1	3	2	-	1
08475	Chile	7	1	3	2	1	1	2	2	1
08484	Brazil	7	1	3	2	1	1	2	-	1
08578	Brazil	7	1	3	2	1	1	2	-	1
08579	Brazil	7	1	3	2	1	1	2	-	1
08580	Brazil	7	1	3	2	1	1	2	2	1
08581	Brazil	7	1	3	2	1	1	2	2	1
08582	Chile	7	1	3	2	1	1	2	2	1
08583	Chile	7	1	3	2	1	1	2	2	1
08584	Chile	7	1	3	1	1	2	2	2	1
08585	Chile	7	1	3	2	1	1	2	2	1
08586	Chile	7	1	3	2	1	1	2	2	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
08587	Chile	1	2	1	2	2	1	1	1	3
08742	Russia	1	2	2	2	1	2	2	2	4
08743	Russia	1	2	2	2	1	2	2	2	4
08744	Russia	1	2	2	2	1	2	2	2	4
08745	Russia	1	2	2	2	1	2	2	2	4
08746	Russia	1	2	2	2	1	2	2	2	4
08747	Russia	1	2	1	2	2	1	1	1	3
08748	Russia	1	2	1	2	2	1	2	2	2
08749	Unknown	1	2	2	2	1	2	2	2	4
08750	Unknown	1	2	2	2	1	2	2	2	4
08751	Unknown	1	2	2	2	1	2	2	2	4
08752	Unknown	1	2	2	2	1	2	2	2	4
08753	Unknown	1	2	1	2	2	1	2	2	2
08754	Unknown	1	2	2	2	1	2	2	2	4
08755	Unknown	1	2	2	2	1	2	2	2	4
08756	Unknown	1	2	2	2	1	2	2	2	4
08757	Unknown	1	2	2	2	2	1	2	2	2
08758	Unknown	1	2	2	2	1	2	2	2	4
08759	Unknown	1	2	2	2	1	2	2	2	4
08760	Unknown	1	2	2	2	1	2	2	2	4
08761	Unknown	1	2	1	2	2	1	2	2	2
08762	Unknown	1	2	1	2	2	1	2	2	2
08763	Unknown	1	2	1	2	2	1	2	2	2
08764	Unknown	1	2	2	2	1	2	2	2	4
08765	Unknown	1	2	2	2	1	2	2	2	4
09440	Germany	1	2	1	2	2	1	1	1	3
09775	Spain	1	2	2	2	1	2	2	2	4
09776	Spain	1	2	2	2	1	2	2	2	4
09778	Morocco	1	2	1	2	2	1	2	2	2
09779	Spain	1	2	1	2	2	1	2	2	2
09781	Portugal	1	2	2	2	1	2	2	2	4

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
08587	Chile	2	2	2	2	2	1	1	2	1
08742	Russia	1	1	2	2	2	1	2	2	4
08743	Russia	1	1	2	2	2	1	2	2	4
08744	Russia	1	1	2	2	2	1	2	2	4
08745	Russia	1	1	2	2	2	1	2	2	13
08746	Russia	1	1	2	2	2	1	2	1	13
08747	Russia	2	2	2	2	2	1	1	2	1
08748	Russia	2	2	2	2	2	1	2	1	3
08749	Unknown	1	1	2	2	2	1	2	1	4
08750	Unknown	1	1	2	2	2	1	2	1	4
08751	Unknown	1	1	2	2	2	1	2	1	4
08752	Unknown	1	1	2	2	2	1	2	1	4
08753	Unknown	2	2	2	2	2	1	1	2	3
08754	Unknown	1	1	2	2	2	1	2	2	4
08755	Unknown	1	1	2	2	2	1	2	2	4
08756	Unknown	1	1	2	2	2	1	2	2	4
08757	Unknown	2	2	2	2	2	1	1	2	3
08758	Unknown	1	1	2	2	2	1	2	1	4
08759	Unknown	1	1	2	2	2	1	2	1	4
08760	Unknown	3	1	2	2	2	1	2	1	4
08761	Unknown	2	2	2	2	2	1	2	1	3
08762	Unknown	2	2	2	2	2	1	2	1	3
08763	Unknown	2	2	2	2	2	1	2	1	3
08764	Unknown	1	1	2	2	2	1	2	1	4
08765	Unknown	1	1	2	2	2	1	2	1	4
09440	Germany	2	2	2	2	2	1	1	2	1
09775	Spain	1	1	2	2	2	1	2	1	4
09776	Spain	1	1	2	2	2	1	2	1	4
09778	Morocco	2	1	2	2	2	1	2	1	3
09779	Spain	2	2	2	2	2	1	2	1	3
09781	Portugal	1	1	2	2	2	1	2	1	4

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
08587	Chile	7	1	3	2	1	1	2	2	1
08742	Russia	1	1	3	3	3	2	1	1	1
08743	Russia	1	1	3	3	3	2	1	1	1
08744	Russia	1	1	3	3	3	2	1	1	1
08745	Russia	2	1	3	3	3	2	1	1	1
08746	Russia	2	1	3	3	3	2	1	1	1
08747	Russia	7	1	3	2	1	1	2	2	1
08748	Russia	6	1	1	1	1	3	2	2	1
08749	Unknown	2	1	3	3	3	2	1	1	1
08750	Unknown	1	1	3	3	3	2	1	1	1
08751	Unknown	1	1	3	3	3	2	1	1	1
08752	Unknown	1	1	3	3	3	2	1	1	1
08753	Unknown	7	1	3	1	1	3	2	2	1
08754	Unknown	1	1	3	3	3	1	1	1	1
08755	Unknown	1	1	3	3	3	1	1	1	1
08756	Unknown	1	1	3	3	3	1	1	1	1
08757	Unknown	7	1	3	1	1	3	2	-	1
08758	Unknown	1	1	3	3	3	2	1	1	1
08759	Unknown	1	1	3	3	3	2	1	1	1
08760	Unknown	1	1	3	3	3	2	1	1	1
08761	Unknown	6	1	1	1	1	3	2	2	1
08762	Unknown	6	1	1	1	1	3	2	2	1
08763	Unknown	1	1	1	1	1	3	2	2	2
08764	Unknown	1	1	3	3	3	2	1	1	1
08765	Unknown	1	1	3	3	3	2	1	1	1
09440	Germany	7	1	3	2	1	1	2	2	1
09775	Spain	1	1	3	3	3	2	2	-	1
09776	Spain	1	1	3	3	3	2	1	1	1
09778	Morocco	6	1	1	2	1	3	2	2	1
09779	Spain	6	1	1	1	1	3	2	-	1
09781	Portugal	1	1	3	3	3	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
09786	Morocco	1	2	1	2	2	3	2	2	7
09791	Morocco	1	2	2	2	1	2	2	2	4
09792	Morocco	1	2	1	2	2	3	2	2	7
09794	Spain	1	2	2	2	1	2	2	2	4
09799	Portugal	1	2	1	2	2	1	2	2	2
09801	Portugal	1	2	1	2	2	3	2	2	7
09804	Portugal	1	2	2	2	1	2	2	2	4
09807	Morocco	1	2	1	2	2	1	2	2	2
09808	Morocco	1	2	1	2	2	3	2	2	7
09809	Portugal	1	2	1	2	2	3	2	2	7
09810	Morocco	1	2	2	2	1	2	1	2	4
09811	Morocco	1	2	1	2	2	3	2	2	7
09814	Spain	1	2	1	2	2	3	2	2	7
09816	Spain	1	2	1	2	2	3	2	2	7
09819	Spain	1	2	2	2	1	2	2	2	3
09821	Morocco	1	2	1	2	2	3	2	2	7
09827	Spain	1	2	1	2	2	3	2	2	7
09830	Spain	1	2	1	2	2	3	2	2	7
09831	Spain	1	2	1	2	2	1	1	1	3
09832	Spain	1	2	1	2	2	1	1	1	3
09833	Spain	2	2	1	2	2	1	1	1	3
09834	Spain	1	2	1	2	2	1	1	1	2
09835	Spain	1	2	1	2	2	1	1	1	2
09836	Spain	1	2	2	2	1	2	2	2	3
09837	Spain	1	2	1	2	2	3	2	2	7
10113	Brazil	1	2	1	2	2	1	1	1	3
10114	Brazil	1	2	1	2	2	1	1	1	3
10118	America	1	2	1	2	2	1	2	2	2
10119	America	1	2	1	2	2	3	2	2	7
10120	America	1	2	1	2	2	1	2	2	2
8482	Unknown	1	2	2	2	1	2	2	2	4

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
09786	Morocco	3	2	2	2	2	1	2	1	2
09791	Morocco	1	1	2	2	2	1	2	1	4
09792	Morocco	3	2	2	2	2	1	2	1	2
09794	Spain	1	1	2	2	2	1	2	1	4
09799	Portugal	2	2	2	2	2	1	2	1	3
09801	Portugal	3	2	2	2	2	1	2	1	2
09804	Portugal	1	1	2	2	2	1	2	1	4
09807	Morocco	2	2	2	2	2	1	2	1	3
09808	Morocco	3	2	2	2	2	1	2	1	2
09809	Portugal	3	1	2	2	2	1	2	1	2
09810	Morocco	1	1	2	2	2	1	2	1	4
09811	Morocco	3	2	2	2	2	1	2	1	2
09814	Spain	3	2	2	2	2	1	2	1	2
09816	Spain	3	2	2	2	2	1	2	1	2
09819	Spain	1	1	2	2	2	1	2	1	4
09821	Morocco	3	2	2	2	2	1	2	1	2
09827	Spain	3	2	2	2	2	1	2	1	2
09830	Spain	3	2	2	2	2	1	2	1	2
09831	Spain	2	2	2	2	2	1	1	2	0
09832	Spain	2	2	2	2	2	1	1	2	0
09833	Spain	2	2	2	2	2	1	1	2	0
09834	Spain	2	2	2	2	2	1	1	2	0
09835	Spain	2	2	2	2	2	1	1	2	0
09836	Spain	1	1	2	2	2	1	2	1	4
09837	Spain	3	2	2	2	2	1	2	1	2
10113	Brazil	2	2	2	2	2	1	1	1	1
10114	Brazil	2	2	2	2	2	1	1	2	1
10118	America	2	2	2	2	2	1	2	1	3
10119	America	-	2	2	2	2	1	2	1	2
10120	America	2	2	2	2	2	1	2	1	7
8482	Unknown	1	1	2	2	2	1	2	1	13

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
09786	Morocco	6	1	2	3	2	6	1	1	1
09791	Morocco	6	1	3	3	3	2	1	1	1
09792	Morocco	6	1	2	3	2	6	1	1	1
09794	Spain	1	1	3	3	3	2	1	1	1
09799	Portugal	6	1	1	1	1	3	2	2	1
09801	Portugal	6	1	2	3	2	6	1	1	1
09804	Portugal	1	1	3	3	3	2	1	1	1
09807	Morocco	6	1	1	1	1	3	2	-	1
09808	Morocco	6	1	2	3	2	6	1	1	1
09809	Portugal	6	1	2	3	2	6	1	1	1
09810	Morocco	2	1	3	3	3	2	2	1	1
09811	Morocco	6	1	2	3	2	6	1	1	1
09814	Spain	6	1	2	3	2	6	1	1	1
09816	Spain	6	1	2	3	2	6	1	1	1
09819	Spain	1	1	4	3	3	2	1	1	1
09821	Morocco	6	1	2	3	2	6	1	1	1
09827	Spain	6	1	2	3	2	6	1	1	1
09830	Spain	6	1	2	3	2	6	1	1	1
09831	Spain	7	1	3	2	1	1	2	2	1
09832	Spain	7	1	3	2	1	1	2	2	1
09833	Spain	7	1	3	2	1	1	2	2	1
09834	Spain	7	-	3	-	1	1	2	2	1
09835	Spain	7	1	3	2	1	1	2	2	1
09836	Spain	1	1	3	3	3	2	1	1	1
09837	Spain	6	1	2	-	2	6	1	1	1
10113	Brazil	7	1	3	2	1	1	2	-	1
10114	Brazil	7	1	3	2	1	1	2	-	1
10118	America	6	1	1	1	1	3	2	-	1
10119	America	6	1	2	3	2	6	1	1	1
10120	America	6	1	1	1	1	3	2	2	1
8482	Unknown	1	1	3	3	3	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
8483	Unknown	1	2	1	2	2	1	1	1	3
8643	Unknown	1	2	1	2	2	1	2	2	2
8644	Unknown	1	2	1	2	2	1	2	2	2
8647	Unknown	1	2	1	2	2	1	2	2	2
8648	Unknown	1	2	1	2	2	1	2	2	2
8649	Unknown	1	2	1	2	2	1	2	2	2
8650	Unknown	1	2	1	2	2	1	2	2	2
8651	Unknown	1	2	1	2	2	1	2	2	2
8654	Unknown	1	2	1	2	2	1	2	2	2
8655	Unknown	1	2	1	2	2	1	2	2	2
8658	Unknown	1	2	2	2	1	2	2	2	4
8659	Unknown	1	2	1	2	2	1	2	2	2
8660	Unknown	1	2	1	2	2	1	2	2	2
8661	Unknown	1	2	1	2	2	1	2	2	2
Cavmen	Australia	1	2	1	2	2	1	1	1	3
Cavmen Pavent	Australia	1	2	1	2	2	1	1	1	3
200 SM1 Barley 3002	Unknown	1	2	1	2	2	1	2	2	2
Fit39eald	Australia	1	2	1	2	2	3	2	2	7
Gairde	Australia	1	2	1	2	2	3	2	2	7
Hindmarsh	Australia	1	2	1	2	2	3	2	2	7
Onslow	Australia	1	2	1	2	2	3	2	2	7
keel	Australia	1	2	2	2	1	2	2	2	4
Maritime	Australia	1	2	1	2	2	1	2	2	2
Flagship	Australia	1	2	2	2	1	2	2	2	4
Doolup	Australia	1	2	2	2	1	2	2	2	4
Fleet	Australia	1	2	1	2	2	3	2	2	7
Haningloh	Canada	1	2	2	2	1	2	2	2	4
Stirling	Australia	1	2	2	2	1	2	2	2	4
Buloke	Australia	1	2	1	2	2	1	1	1	3
Hamelin(09 WH P3)	Australia	1	2	2	2	1	2	2	2	5
Viamingh(06 NH M4)	Australia	1	2	2	2	1	2	2	2	5

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
8483	Unknown	2	2	2	2	2	1	1	2	1
8643	Unknown	2	-	2	2	2	1	1	1	3
8644	Unknown	2	-	2	2	2	1	1	1	3
8647	Unknown	2	2	2	2	2	1	1	1	3
8648	Unknown	2	2	2	2	2	1	1	1	3
8649	Unknown	2	2	2	2	2	1	1	1	3
8650	Unknown	2	2	2	2	2	1	1	2	3
8651	Unknown	2	2	2	2	2	1	1	2	3
8654	Unknown	2	2	2	2	2	1	1	2	3
8655	Unknown	2	2	2	2	2	1	1	2	3
8658	Unknown	1	1	2	2	2	1	2	1	4
8659	Unknown	2	2	2	2	2	1	1	2	3
8660	Unknown	2	2	2	2	2	1	1	2	3
8661	Unknown	2	-	2	2	2	1	1	1	3
Cavmen	Australia	2	2	2	2	2	1	1	2	-
Cavmen Pavent	Australia	2	2	2	2	2	1	1	2	1
200 SM1 Barley 3002	Unknown	2	2	2	2	2	1	2	1	3
Fit39eald	Australia	3	2	2	2	2	1	2	1	2
Gairde	Australia	3	2	2	2	2	1	2	1	2
Hindmarsh	Australia	3	2	2	2	2	1	2	1	2
Onslow	Australia	3	2	2	2	2	1	2	1	2
keel	Australia	1	1	2	2	2	1	2	1	4
Maritime	Australia	2	2	2	2	2	1	1	2	3
Flagship	Australia	1	1	2	2	2	1	2	1	4
Doolup	Australia	1	1	2	2	2	1	2	1	4
Fleet	Australia	3	2	2	2	2	1	2	1	2
Haningloh	Canada	1	1	2	2	2	1	2	1	4
Stirling	Australia	2	1	2	2	2	1	2	1	4
Buloke	Australia	2	2	2	2	2	1	1	2	1
Hamelin(09 WH P3)	Australia	1	1	2	2	2	1	2	1	4
Viamingh(06 NH M4)	Australia	1	1	2	2	2	1	2	1	4

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
8483	Unknown	7	1	3	2	1	1	2	2	1
8643	Unknown	7	1	3	1	1	3	2	2	1
8644	Unknown	7	1	3	1	1	3	2	2	1
8647	Unknown	7	1	3	1	1	3	2	2	1
8648	Unknown	7	1	3	1	1	3	2	2	1
8649	Unknown	7	1	3	1	1	3	2	2	1
8650	Unknown	7	1	3	1	1	3	2	2	1
8651	Unknown	7	1	3	1	1	3	2	2	1
8654	Unknown	7	1	3	1	1	3	2	2	1
8655	Unknown	7	1	3	1	1	3	2	2	1
8658	Unknown	1	1	3	3	3	2	1	1	1
8659	Unknown	7	1	3	3	1	3	2	2	1
8660	Unknown	7	1	3	3	1	3	2	2	1
8661	Unknown	7	1	3	1	1	3	2	2	1
Cavmen	Australia	7	1	3	-	1	1	2	2	1
Cavmen Pavent	Australia	7	1	3	2	1	1	2	-	1
200 SM1 Barley 3002	Unknown	6	1	1	1	1	3	2	2	1
Fit39eald	Australia	6	1	2	1	2	6	1	1	1
Gairde	Australia	6	1	2	3	2	6	1	1	1
Hindmarsh	Australia	6	1	2	3	2	6	1	1	1
Onslow	Australia	6	1	2	3	2	6	1	1	1
keel	Australia	2	1	1	3	3	2	1	1	1
Maritime	Australia	7	1	1	1	1	3	2	2	1
Flagship	Australia	1	1	1	3	3	2	1	1	1
Doolup	Australia	1	1	3	3	3	2	1	1	1
Fleet	Australia	6	1	2	3	2	6	1	1	1
Haningloh	Canada	1	1	2	3	3	2	1	1	1
Stirling	Australia	1	1	3	3	3	2	1	1	1
Buloke	Australia	7	1	3	2	1	1	2	-	1
Hamelin(09 WH P3)	Australia	2	1	3	3	3	-	1	1	1
Viamingh(06 NH M4)	Australia	2	1	3	3	3	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
Mundah	Australia	1	2	1	2	2	3	2	2	7
Baudin	Australia	1	2	2	2	1	2	2	2	4
WABAR2473 (06 WH M3)	Australia	1	2	2	2	1	2	2	2	4
WABAR2473(07 WH P1)	Australia	1	2	2	2	1	2	2	2	4
XZ76	China	1	2	2	2	1	2	2	2	4
XZ54/GG1	China	1	2	2	2	1	2	2	2	4
Sook Awn 86	America	1	2	1	2	2	3	2	2	2
B501	China	1	2	2	2	1	2	2	2	5
B503	China	1	2	2	2	1	2	2	2	5
B515	China	1	2	2	2	1	2	2	2	4
B521	China	1	2	2	2	1	2	2	2	4
B523	China	1	2	2	2	1	2	2	2	4
B531	China	1	2	2	2	1	2	2	2	4
B537	China	1	2	2	2	1	2	2	2	4
B539	China	1	2	2	2	1	2	2	2	4
B557	China	1	2	2	2	1	2	2	2	4
B559	China	1	2	2	2	1	2	2	2	4
B561	China	1	2	2	2	1	2	2	2	4
B575	China	1	2	2	2	1	2	2	2	4
B577	China	1	2	2	2	1	2	2	2	4
B581	China	1	2	2	2	1	2	2	2	4
B583	China	1	2	2	2	1	2	2	2	4
B585	China	1	2	2	2	1	2	2	2	4
B587	China	1	2	1	2	2	1	2	2	2
B591	China	1	2	1	2	2	3	2	2	7
B611	China	1	2	2	2	1	2	2	2	4
B615	China	1	2	2	2	1	2	2	2	4
B617	China	1	2	2	2	1	2	2	2	4
B627	China	1	2	2	2	1	2	2	2	4
B645	China	1	2	2	2	1	2	2	2	4
B653	China	1	2	1	2	2	1	2	2	2

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
Mundah	Australia	3	2	2	2	2	1	2	1	2
Baudin	Australia	1	1	2	2	2	1	2	1	4
WABAR2473 (06 WH M3)	Australia	1	1	2	2	2	1	2	1	4
WABAR2473(07 WH P1)	Australia	1	1	2	2	2	1	2	1	4
XZ76	China	1	1	2	2	2	1	2	1	4
XZ54/GG1	China	1	1	2	2	2	1	2	1	4
Sook Awn 86	America	-	2	2	2	2	1	2	1	3
B501	China	1	1	2	2	2	1	2	1	4
B503	China	1	1	2	2	2	1	2	1	4
B515	China	1	1	2	2	2	1	2	1	4
B521	China	1	1	2	2	2	1	2	1	4
B523	China	1	1	2	2	2	1	2	1	4
B531	China	1	1	2	2	2	1	2	1	4
B537	China	1	1	2	2	2	1	2	1	4
B539	China	1	1	2	2	2	1	2	1	4
B557	China	1	1	2	2	2	1	2	2	4
B559	China	1	1	2	2	2	1	2	1	4
B561	China	1	1	2	2	2	1	2	1	4
B575	China	1	1	2	2	2	1	2	1	4
B577	China	1	1	2	2	2	1	2	1	4
B581	China	1	1	2	2	2	1	2	1	-
B583	China	1	1	2	2	2	1	2	1	4
B585	China	1	1	2	2	2	1	2	1	4
B587	China	2	2	2	2	2	1	2	1	3
B591	China	3	2	2	2	2	1	2	1	2
B611	China	1	1	2	2	2	1	2	1	4
B615	China	1	1	2	2	2	1	2	1	4
B617	China	1	1	2	2	2	1	2	1	4
B627	China	1	1	2	2	2	1	2	1	4
B645	China	1	1	2	2	2	1	2	1	4
B653	China	2	2	2	2	2	1	2	1	3

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
Mundah	Australia	6	1	2	3	2	6	1	1	1
Baudin	Australia	2	1	3	3	3	2	1	1	1
WABAR2473 (06 WH M3)	Australia	2	2	3	3	3	2	1	1	1
WABAR2473(07 WH P1)	Australia	1	1	3	3	3	2	1	1	1
XZ76	China	2	1	3	3	3	2	1	1	1
XZ54/GG1	China	2	1	3	3	3	2	1	1	1
Sook Awn 86	America	6	1	1	1	1	3	2	-	2
B501	China	2	1	3	-	3	2	1	1	1
B503	China	2	1	3	3	3	2	1	1	1
B515	China	1	1	3	3	3	2	1	1	1
B521	China	1	1	3	3	3	2	1	1	1
B523	China	1	1	3	3	8	2	1	1	1
B531	China	1	1	3	3	3	2	1	1	1
B537	China	1	1	3	3	3	2	1	1	1
B539	China	1	1	3	3	3	2	1	1	1
B557	China	1	1	3	3	3	2	1	1	1
B559	China	1	1	3	3	3	2	1	1	1
B561	China	1	1	3	3	3	2	1	1	1
B575	China	1	1	3	3	3	2	1	1	1
B577	China	1	1	3	3	3	2	1	1	1
B581	China	1	1	3	3	3	2	1	1	1
B583	China	1	1	3	3	3	2	1	1	1
B585	China	1	1	3	3	3	2	1	1	1
B587	China	6	1	1	1	1	3	2	-	1
B591	China	6	1	2	3	2	1	1	1	1
B611	China	1	1	3	3	3	2	1	1	1
B615	China	1	1	3	3	3	2	1	1	1
B617	China	1	1	3	3	3	2	1	1	1
B627	China	1	1	3	3	3	2	1	1	1
B645	China	1	1	3	3	3	2	1	1	1
B653	China	6	1	5	1	1	3	1	-	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
B657	China	1	2	2	2	1	2	2	2	4
B659	China	1	2	2	2	1	2	2	2	4
B671	China	1	2	2	2	1	2	2	2	4
B685	China	1	2	2	2	1	2	2	2	4
B689	China	1	2	1	2	2	1	2	2	2
B695	China	1	2	2	2	1	2	2	2	4
B701	China	1	2	2	2	1	2	2	2	4
B703	China	1	2	2	2	1	2	2	2	4
B705	China	1	2	2	2	1	2	2	2	4
B751	China	1	2	1	2	2	1	1	1	3
B759	China	1	2	1	2	2	1	1	1	1
B769	China	1	2	2	2	1	2	1	2	5
B771	China	1	2	2	2	1	2	2	2	4
B779	China	1	2	1	2	2	1	1	1	3
B783	China	1	2	2	2	1	2	2	2	4
B797	China	1	2	1	2	2	1	2	2	2
X51	China	1	2	3	2	2	2	1	2	9
X97	China	1	2	2	2	1	2	2	2	4
X112	China	1	2	2	2	1	2	2	2	4
X117	China	1	2	1	2	2	1	2	2	4
X153	China	1	2	1	2	2	1	2	2	2
X160	China	1	2	1	2	2	1	2	2	3
X161	China	1	2	1	2	2	1	2	2	2
KM 123	China	1	2	1	2	2	1	1	1	3
Z034P116Q	China	1	2	1	2	2	1	1	2	2
Z035R014S	China	1	2	2	2	1	2	2	2	4
WVB 29	Unknown	1	2	2	2	1	2	2	2	4
WVB 33	Unknown	1	2	2	-	1	2	2	2	4
H95030001	Canada	1	2	2	2	1	2	2	2	4
H95039003	Canada	1	2	2	2	1	2	2	2	4
Cevada de 6 Ordens	Portugal	1	2	1	2	2	3	2	2	7

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
B657	China	1	1	2	2	2	1	2	1	4
B659	China	1	1	2	2	2	1	2	1	4
B671	China	1	1	2	2	2	1	2	1	4
B685	China	1	1	2	2	2	1	2	1	4
B689	China	2	2	2	2	2	1	2	1	3
B695	China	1	1	2	2	2	1	2	1	4
B701	China	1	1	2	2	2	1	2	1	4
B703	China	1	1	2	2	2	1	2	1	4
B705	China	1	1	2	2	2	1	2	1	4
B751	China	2	2	2	2	2	1	1	2	1
B759	China	2	2	2	2	2	1	1	2	15
B769	China	1	1	2	2	2	1	2	1	4
B771	China	1	1	2	2	2	1	2	1	4
B779	China	2	2	2	2	2	1	1	2	1
B783	China	1	1	2	2	2	1	2	1	4
B797	China	2	2	2	2	2	1	2	1	3
X51	China	5	2	2	2	3	3	2	2	11
X97	China	1	1	2	2	2	1	2	1	4
X112	China	1	1	2	2	2	1	2	1	4
X117	China	2	2	2	2	1	2	2	2	3
X153	China	2	2	2	2	2	1	2	1	3
X160	China	2	2	2	2	1	2	2	2	3
X161	China	2	2	2	2	2	1	2	1	3
KM 123	China	2	2	2	2	2	1	1	2	1
Z034P116Q	China	2	2	2	2	2	1	1	2	5
Z035R014S	China	1	1	2	2	2	1	2	2	4
WVB 29	Unknown	1	1	2	2	2	1	2	2	4
WVB 33	Unknown	1	1	2	2	2	1	2	2	4
H95030001	Canada	1	1	2	2	2	1	2	2	4
H95039003	Canada	1	1	2	2	2	1	2	2	4
Cevada de 6 Ordens	Portugal	3	2	2	2	2	1	2	2	2

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
B657	China	1	1	3	3	3	2	1	1	1
B659	China	1	1	3	3	3	2	1	1	1
B671	China	1	1	3	3	3	2	1	1	1
B685	China	1	1	3	3	3	2	1	1	1
B689	China	6	1	1	1	1	3	1	2	1
B695	China	1	1	3	3	3	2	1	1	1
B701	China	1	1	3	3	3	2	1	1	1
B703	China	1	1	3	3	3	2	1	1	1
B705	China	1	1	3	3	3	2	1	1	1
B751	China	7	1	3	2	1	1	2	-	1
B759	China	7	1	3	2	1	1	2	-	1
B769	China	1	1	3	3	3	2	1	1	1
B771	China	1	1	3	3	3	2	1	1	1
B779	China	7	1	3	2	1	1	2	2	1
B783	China	1	1	3	3	3	2	1	1	1
B797	China	6	1	5	1	1	3	1	-	1
X51	China	3	1	3	5	-	3	1	1	1
X97	China	1	1	3	3	3	2	1	1	1
X112	China	1	1	3	3	3	2	1	1	1
X117	China	2	1	3	2	1	3	2	2	1
X153	China	6	1	1	1	1	3	2	-	1
X160	China	2	1	3	2	1	3	2	-	1
X161	China	6	1	1	1	1	3	2	-	1
KM 123	China	7	1	3	2	1	-	1	-	1
Z034P116Q	China	7	1	3	1	1	3	1	2	1
Z035R014S	China	2	1	3	3	3	2	1	1	-
WVB 29	Unknown	2	1	3	3	3	2	1	1	-
WVB 33	Unknown	2	1	3	3	3	2	1	1	1
H95030001	Canada	2	1	3	3	8	2	1	1	1
H95039003	Canada	2	1	4	3	3	2	1	1	1
Cevada de 6 Ordens	Portugal	6	1	2	3	2	6	2	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
Waggon	The United Kingdom	1	2	2	2	1	-	2	2	4
Cocktail	The European Union	1	2	2	2	1	2	2	2	4
H95011024	Canada	1	2	1	2	2	1	1	1	3
H95056002	Canada	1	2	1	2	2	1	1	1	3
Keka	Spain	1	2	1	2	2	-	1	2	11
Rosa	Unknown	1	2	1	2	2	-	1	1	4
Gairdner	Australia	1	2	1	2	2	3	2	2	7
Gairdner	Australia	1	2	1	2	2	3	2	2	7
BM9919-90	Canada	1	2	2	2	1	2	2	2	7
2B03-3604	Unknown	1	2	2	2	1	2	2	2	4
WVA 18	Unknown	1	2	1	2	2	3	2	2	7
WVA 19	Unknown	1	2	2	2	1	2	2	2	4
Wicket	The United Kingdom	1	2	2	2	1	2	2	2	4
Flagon	Unknown	1	2	2	2	1	1	2	2	4
H95056005	Canada	1	2	1	2	2	1	1	1	3
Adagio	Unknown	1	2	1	2	2	1	2	2	2
Moroccan Landrace	Morocco	1	2	1	2	2	3	2	2	7
Spanish - 309d	Spain	1	2	1	2	2	3	2	2	7
Hamelin	Australia	1	2	2	2	1	2	2	2	4
Hamelin	Australia	1	2	2	2	1	2	2	2	4
2B03-3631	Unknown	1	2	2	2	1	2	2	2	4
2B03-3785	Unknown	1	2	2	2	1	2	2	2	4
WVA 20	Unknown	1	2	2	2	1	2	2	2	4
WVA 22	Unknown	1	2	2	2	1	2	2	2	4
Braemer	Germany	-	2	2	2	1	2	2	2	4
H95027004	Canada	1	2	1	2	1	1	2	2	4
Braemar	Germany	1	2	2	2	1	2	2	2	4
HOR12517	Spain	1	2	2	2	1	2	2	2	4
Spanish Landrace-316	Spain	1	2	1	2	1	3	2	2	7
Spanish Landrace-333c	Spain	-	2	2	2	1	2	2	2	3
Stirling	Australia	1	2	2	2	1	2	2	2	4

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
Waggon	The United Kingdom	1	1	2	2	2	1	2	1	4
Cocktail	The European Union	1	1	2	2	2	1	2	1	4
H95011024	Canada	2	-	2	2	2	1	1	1	-
H95056002	Canada	2	2	2	2	2	1	1	1	1
Keka	Spain	2	2	2	2	2	1	2	1	2
Rosa	Unknown	2	2	2	2	2	1	1	2	1
Gairdner	Australia	3	2	2	2	2	1	2	1	2
Gairdner	Australia	3	2	2	2	2	1	2	1	2
BM9919-90	Canada	1	1	2	2	2	1	2	1	4
2B03-3604	Unknown	1	1	2	2	2	1	2	1	4
WVA 18	Unknown	3	2	2	2	2	1	2	1	2
WVA 19	Unknown	1	1	2	2	2	1	2	1	4
Wicket	The United Kingdom	1	1	2	2	2	1	2	1	4
Flagon	Unknown	1	1	2	2	2	1	2	1	4
H95056005	Canada	2	2	2	2	2	1	1	1	17
Adagio	Unknown	2	-	2	2	2	1	1	2	18
Moroccan Landrace	Morocco	3	2	2	2	2	1	2	1	2
Spanish - 309d	Spain	3	2	2	2	2	1	2	1	2
Hamelin	Australia	1	1	2	2	2	1	2	1	4
Hamelin	Australia	1	1	2	2	2	1	2	1	4
2B03-3631	Unknown	1	1	2	2	2	1	2	1	4
2B03-3785	Unknown	1	1	2	2	2	1	2	1	4
WVA 20	Unknown	1	1	2	2	2	1	2	1	4
WVA 22	Unknown	1	1	2	2	2	1	2	1	4
Braemer	Germany	-	1	-	2	2	1	2	1	4
H95027004	Canada	2	2	2	2	2	1	1	1	4
Braemar	Germany	1	1	-	2	2	1	2	1	4
HOR12517	Spain	1	1	2	2	2	1	2	1	4
Spanish Landrace-316	Spain	2	2	-	2	2	1	2	1	2
Spanish Landrace-333c	Spain	1	-	2	2	2	1	-	2	5
Stirling	Australia	1	1	2	2	2	1	2	1	4

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
Waggon	The United Kingdom	2	1	3	3	3	2	1	1	1
Cocktail	The European Union	2	1	3	3	3	2	1	1	1
H95011024	Canada	7	1	3	2	1	1	1	1	1
H95056002	Canada	7	1	3	2	1	1	1	1	1
Keka	Spain	6	1	2	3	2	6	2	1	1
Rosa	Unknown	7	1	3	2	1	1	1	1	1
Gairdner	Australia	6	1	2	3	2	6	2	1	1
Gairdner	Australia	6	1	2	3	2	6	2	1	1
BM9919-90	Canada	1	1	3	3	3	2	1	1	1
2B03-3604	Unknown	1	1	3	3	3	2	1	1	1
WVA 18	Unknown	1	1	2	3	2	6	2	1	1
WVA 19	Unknown	1	1	3	3	3	2	1	1	1
Wicket	The United Kingdom	1	1	3	3	3	2	1	1	1
Flagon	Unknown	1	1	3	3	3	2	1	1	1
H95056005	Canada	7	1	3	2	1	1	1	1	1
Adagio	Unknown	7	1	3	1	1	3	2	1	1
Moroccan Landrace	Morocco	6	1	2	-	2	6	2	1	1
Spanish -309d	Spain	6	1	2	3	2	6	2	1	1
Hamelin	Australia	1	1	3	3	3	2	1	1	1
Hamelin	Australia	1	1	3	3	3	2	1	1	1
2B03-3631	Unknown	1	1	3	3	3	2	1	1	1
2B03-3785	Unknown	1	1	3	3	3	2	1	1	1
WVA 20	Unknown	1	1	3	3	3	2	1	1	1
WVA 22	Unknown	1	1	3	3	3	2	1	1	1
Braemer	Germany	1	1	3	3	3	2	1	1	1
H95027004	Canada	7	1	3	1	1	3	1	1	1
Braemar	Germany	1	1	3	-	3	2	1	1	1
HOR12517	Spain	1	1	3	3	3	2	1	1	1
Spanish Landrace-316	Spain	6	1	2	3	2	6	2	1	1
Spanish Landrace-333c	Spain	1	1	3	-	3	2	1	1	1
Stirling	Australia	1	1	3	3	3	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
Stirling	Australia	1	2	2	2	1	2	2	2	4
2B03-3830	Unknown	1	2	2	2	1	2	2	2	4
2B03-3859	Unknown	1	2	2	2	1	2	2	2	4
WVA 24	Unknown	1	2	2	2	1	2	2	2	4
WVB 7	Unknown	1	2	1	2	2	1	1	1	3
H95032005	Canada	1	2	2	2	1	2	2	2	4
H96009015001	Canada	1	2	2	2	1	2	2	2	4
Portuguese Landrace	Portugal	1	2	2	2	1	2	2	-	4
MAR-86-E1138	Unknown	1	2	2	2	1	2	2	2	6
Spanish Landrace-336d	Spain	1	2	1	2	2	1	1	1	3
Spanish Landrace-338c	Spain	2	2	3	2	2	-	1	1	3
Vlamingh	Australia	1	2	2	2	1	2	2	2	5
Vlamingh	Australia	1	2	2	2	1	2	2	2	5
2B03-3882	Unknown	1	2	2	2	1	2	2	2	4
Z034P013Q	China	1	2	1	2	2	1	2	2	2
WVB 9	Unknown	1	2	2	2	1	2	2	2	4
WVB 22	Unknown	1	2	2	2	1	2	2	2	4
H96009015002	Canada	1	2	2	2	1	2	2	2	4
M94060003	Canada	1	2	2	2	1	2	2	2	4
HOR13461	Spain	1	2	2	2	1	2	2	2	4
Cevada de 2 Ordens	Portugal	1	2	1	2	2	1	2	2	2
Spanish Landrace-349	Spain	1	2	1	2	2	1	2	1	3
Spanish Landrace-349b	Spain	1	2	1	2	2	1	1	1	3
WABAR2315	Australia	1	2	2	2	1	2	2	2	5
WABAR2315	Australia	1	2	2	2	1	2	2	2	5
93-3143	China	1	2	2	2	1	2	2	2	4
Aizao 3	China	1	2	2	2	1	2	2	2	4
Lixi 143	China	1	2	2	2	1	2	2	2	4
Naso nijo	Japan	1	2	2	2	1	2	2	2	4
ZUG293	China	1	2	2	2	1	2	2	2	4
ZUG403	China	1	2	3	2	1	2	2	2	4

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
Stirling	Australia	1	1	2	2	2	1	2	1	4
2B03-3830	Unknown	1	1	2	2	2	1	2	1	4
2B03-3859	Unknown	1	1	2	2	2	1	2	1	4
WVA 24	Unknown	1	1	2	2	2	1	2	1	4
WVB 7	Unknown	2	-	2	2	2	1	-	2	17
H95032005	Canada	1	1	2	2	2	1	2	1	4
H96009015001	Canada	1	1	2	2	2	1	2	1	4
Portuguese Landrace	Portugal	1	1	2	2	2	1	2	1	4
MAR-86-E1138	Unknown	1	1	2	2	2	1	2	1	4
Spanish Landrace-336d	Spain	2	2	2	2	2	1	-	2	-
Spanish Landrace-338c	Spain	2	2	2	2	2	1	1	2	8
Vlamingh	Australia	1	1	2	2	2	1	2	2	4
Vlamingh	Australia	1	1	2	2	2	1	2	2	4
2B03-3882	Unknown	1	1	2	2	2	1	2	1	4
Z034P013Q	China	2	2	2	2	2	1	1	2	5
WVB 9	Unknown	1	1	2	2	2	1	2	1	4
WVB 22	Unknown	1	1	2	2	2	1	2	1	4
H96009015002	Canada	1	1	2	2	2	1	2	1	4
M94060003	Canada	1	1	2	2	2	1	2	1	4
HOR13461	Spain	1	-	2	2	2	1	2	1	4
Cevada de 2 Ordens	Portugal	2	2	2	2	2	1	2	1	5
Spanish Landrace-349	Spain	2	2	2	2	2	1	1	2	1
Spanish Landrace-349b	Spain	2	2	2	2	2	1	1	2	1
WABAR2315	Australia	1	1	2	2	2	1	2	1	4
WABAR2315	Australia	1	1	2	2	2	1	2	1	4
93-3143	China	1	1	2	2	2	1	2	1	4
Aizao 3	China	1	1	2	2	2	1	2	1	4
Lixi 143	China	1	1	2	2	2	1	2	1	4
Naso nijo	Japan	1	1	2	2	2	1	2	1	4
ZUG293	China	1	1	2	2	2	1	2	1	4
ZUG403	China	1	1	2	2	2	1	2	1	4

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
Stirling	Australia	1	1	3	3	3	2	1	1	1
2B03-3830	Unknown	1	1	3	3	3	2	1	1	1
2B03-3859	Unknown	1	1	3	3	3	2	1	1	1
WVA 24	Unknown	1	1	3	3	1	2	1	1	1
WVB 7	Unknown	1	1	3	2	-	1	1	1	1
H95032005	Canada	1	1	3	3	3	2	1	1	1
H96009015001	Canada	1	1	3	3	8	2	1	1	1
Portuguese Landrace	Portugal	1	1	3	3	3	2	1	1	1
MAR-86-E1138	Unknown	1	1	3	3	3	2	1	1	1
Spanish Landrace-336d	Spain	7	1	3	2	1	1	1	1	1
Spanish Landrace-338c	Spain	7	1	3	2	1	1	1	1	1
Vlamingh	Australia	2	1	3	3	3	2	1	1	1
Vlamingh	Australia	2	1	3	3	3	2	1	1	1
2B03-3882	Unknown	1	1	3	3	3	2	1	1	1
Z034P013Q	China	7	1	3	1	1	3	1	2	1
WVB 9	Unknown	1	1	3	3	3	2	1	1	1
WVB 22	Unknown	1	1	3	3	3	2	1	1	1
H96009015002	Canada	1	1	3	3	8	2	1	1	1
M94060003	Canada	1	1	3	3	3	2	1	1	1
HOR13461	Spain	1	1	3	3	-	2	1	1	1
Cevada de 2 Ordens	Portugal	6	1	1	1	1	3	1	2	1
Spanish Landrace-349	Spain	7	1	3	2	1	3	1	1	1
Spanish Landrace-349b	Spain	7	1	3	2	1	3	1	1	1
WABAR2315	Australia	2	1	3	3	3	2	1	1	1
WABAR2315	Australia	2	1	3	3	3	2	1	1	1
93-3143	China	1	1	3	3	3	2	1	1	1
Aizao 3	China	1	1	3	3	3	2	1	1	1
Lixi 143	China	1	1	3	3	3	2	1	1	1
Naso nijo	Japan	1	1	3	3	3	2	1	1	1
ZUG293	China	1	1	3	3	3	2	1	1	1
ZUG403	China	1	1	3	3	-	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
YSM3	China	1	2	2	2	1	2	2	2	4
YU6472	China	1	2	2	2	1	2	2	2	4
Brindabella	Australia	1	2	1	2	2	1	2	2	2
CM72	America	1	2	2	2	1	2	2	2	4
Numar	America	1	2	2	2	-	2	2	2	4
RGZLL	Unknown	1	2	1	2	2	1	2	2	2
Barque 73	Australia	1	2	2	2	1	2	2	2	4
Clipper	Australia	1	2	2	2	1	2	2	2	4
CPI-11284-48	Unknown	1	2	1	2	2	3	2	2	4
CxHKSL	China	1	2	1	2	2	1	2	2	2
SYR01	Syria	1	1	1	2	2	5	2	2	4
AC Burman	Canada	1	2	2	2	1	2	2	2	4
Flagship	Australia	1	2	2	2	1	2	2	2	4
Keel	Australia	1	2	2	2	1	2	2	2	4
Dash	Australia	1	2	2	2	1	2	2	2	4
Dayton	Australia	1	2	1	2	2	1	2	2	2
Macquarie	Australia	1	2	1	2	2	3	2	2	7
TF026	Australia	1	2	2	2	1	2	2	2	4
Mundah	Australia	1	2	1	2	1	3	2	2	7
Sahara	Africa	1	2	2	2	1	1	2	2	4
DYSYH	China	1	2	3	2	2	6	2	2	8
Franklin	Australia	1	2	3	2	2	6	3	3	9
YF374	China	1	2	2	2	1	2	2	2	4
Tx9425	China	1	2	2	2	1	2	2	2	4
Schooner	Australia	1	2	2	2	1	2	2	2	4
Skiff	Australia	1	2	2	2	1	2	2	2	4
Gairdner	Australia	1	2	1	2	2	3	2	2	7
Gebeina	Germany	1	2	2	2	1	2	2	2	4
Unicorn	Japan	1	2	2	2	1	2	2	2	4
Xiaojiang	China	1	2	2	2	1	2	2	2	4
Yan89110	China	1	2	2	2	1	2	2	2	4

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
YSM3	China	1	1	2	2	2	1	2	1	4
YU6472	China	1	1	2	2	2	1	2	-	4
Brindabella	Australia	2	2	2	2	2	1	1	2	5
CM72	America	1	1	2	2	2	1	2	1	4
Numar	America	1	1	2	2	2	1	2	1	4
RGZLL	Unknown	2	2	2	2	2	1	2	1	5
Barque 73	Australia	1	1	2	2	2	1	2	1	17
Clipper	Australia	1	1	2	2	2	1	2	1	4
CPI-11284-48	Unknown	3	2	2	2	2	1	2	2	2
CxHKSL	China	2	2	2	2	2	1	-	1	5
SYR01	Syria	3	2	1	3	2	1	2	2	4
AC Burman	Canada	1	1	2	2	2	1	2	1	4
Flagship	Australia	1	1	2	2	2	1	2	1	4
Keel	Australia	1	1	2	2	2	1	2	1	4
Dash	Australia	1	1	2	2	2	1	2	1	4
Dayton	Australia	2	2	2	2	2	1	2	1	5
Macquarie	Australia	3	2	2	2	2	1	2	1	2
TF026	Australia	1	1	2	2	2	1	2	1	4
Mundah	Australia	3	2	2	2	2	1	2	1	2
Sahara	Africa	1	1	2	2	2	1	2	1	4
DYSYH	China	5	2	2	2	2	1	2	1	4
Franklin	Australia	5	2	2	2	2	1	3	2	4
YF374	China	1	1	2	2	2	1	2	1	4
Tx9425	China	-	1	2	2	2	1	2	2	4
Schooner	Australia	1	1	2	2	2	1	2	2	4
Skiff	Australia	1	1	2	2	-	1	2	2	4
Gairdner	Australia	3	2	2	2	2	1	2	1	2
Gebeina	Germany	1	1	2	2	2	1	2	1	4
Unicorn	Japan	1	1	2	2	2	1	2	1	4
Xiaojiang	China	1	1	2	2	2	1	2	2	4
Yan89110	China	1	1	2	2	2	1	2	2	4

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2F
YSM3	China	1	1	3	3	3	2	1	1	1
YU6472	China	1	1	-	3	3	2	1	1	1
Brindabella	Australia	7	1	3	1	1	3	2	2	1
CM72	America	1	1	3	3	3	2	1	1	1
Numar	America	1	1	3	3	3	2	1	1	1
RGZLL	Unknown	6	1	-	1	1	-	2	2	1
Barque 73	Australia	4	1	3	3	3	2	1	1	1
Clipper	Australia	1	1	3	3	-	2	1	1	1
CPI-11284-48	Unknown	2	3	3	-	-	1	1	-	1
CxHKSL	China	6	1	1	1	1	3	2	2	1
SYR01	Syria	2	1	3	3	2	3	1	1	1
AC Burman	Canada	1	1	3	3	3	2	1	1	1
Flagship	Australia	1	1	3	3	3	2	1	1	1
Keel	Australia	1	1	3	3	3	2	1	1	1
Dash	Australia	1	1	3	3	3	2	1	1	1
Dayton	Australia	6	1	1	1	1	3	2	2	2
Macquarie	Australia	6	1	3	3	2	1	1	1	1
TF026	Australia	1	1	3	3	3	2	1	1	1
Mundah	Australia	6	1	3	3	1	1	1	1	1
Sahara	Africa	1	1	3	3	3	2	1	1	1
DYSYH	China	4	1	6	6	1	3	1	1	1
Franklin	Australia	5	1	3	5	-	4	1	1	1
YF374	China	1	1	3	3	3	2	1	1	1
Tx9425	China	1	1	3	3	3	2	1	1	1
Schooner	Australia	1	1	3	3	3	2	1	1	1
Skiff	Australia	1	2	3	3	3	2	1	1	1
Gairdner	Australia	6	1	3	3	3	6	1	1	1
Gebeina	Germany	1	1	3	3	3	2	1	1	1
Unicorn	Japan	1	1	3	3	3	2	1	1	1
Xiaojiang	China	1	1	3	3	3	2	1	1	1
Yan89110	China	1	1	3	3	3	2	1	1	1

Table A-2 (continued)

Acession	Origion	Cit6	Cit18	Cit7	Cit3	Cit14	Cit16	Cit1001	Cit9	D202RR
Yan90260	China	1	2	1	2	2	1	1	1	3
Haruna nijo	Japan	1	2	2	2	1	2	2	2	4
Honen	Japan	1	2	1	2	2	1	2	2	2
YUQS	China	1	2	2	2	1	2	2	-	4
YWHKSL	China	1	2	1	2	2	1	2	2	2
Yerong	China	1	2	2	2	1	2	2	2	4
Yiwu Erleng	China	1	2	1	2	1	1	2	2	2
Hu93-043	China	1	2	2	2	1	2	2	2	4
Kinu nijo 6	Japan	1	2	2	2	1	2	2	2	4
YYXT	China	1	2	2	2	1	2	2	2	4
Zhepi 2	China	1	2	2	2	1	2	2	2	4
YPSLDM	China	1	2	1	2	2	1	2	2	2
YSMI	China	1	2	1	2	2	1	2	2	2
W2	Tibet	1	2	2	2	1	2	2	2	4
BR2	Brazil	1	2	1	2	2	1	2	2	2
Hamelin	Australia	1	2	2	2	1	2	2	2	4
Svanhals	CYMMIT	1	2	1	2	2	1	1	1	3
Yambla	China	1	2	1	2	2	1	1	1	3
PTWOOM-2	China	1	2	1	2	-	1	2	2	2
PTWOOM-3	China	1	2	1	2	2	1	2	2	2
PTWOOM-4	China	1	2	1	2	2	1	2	2	2
PTWOOM-5	China	1	2	1	2	2	1	2	2	2
PTWOOM-6	China	1	2	1	2	2	1	2	2	2
PTWOOM-8	China	1	2	1	2	2	1	2	2	2
Pavlovicky	Russia	-	-	-	-	2	-	-	-	7

Table A-2 (continued)

Acession	Origion	Cit1501	Cit19	Cit501	Cit12	Cit0	Cit1	U12F+U11R	U5	D4FF
Yan90260	China	2	2	2	2	2	1	1	2	2
Haruna nijo	Japan	1	1	2	2	2	1	2	1	4
Honen	Japan	2	2	2	2	2	1	2	1	5
YUQS	China	1	1	2	2	2	1	2	1	4
YWHKSL	China	-	2	2	2	2	1	2	1	5
Yerong	China	1	1	2	2	2	1	2	1	4
Yiwu Erleng	China	2	2	2	2	2	1	2	1	4
Hu93-043	China	1	1	2	2	2	1	2	1	4
Kinu nijo 6	Japan	1	1	2	2	2	1	2	1	4
YYXT	China	1	1	2	2	2	1	2	1	4
Zhepi 2	China	1	1	2	2	2	1	2	1	4
YPSLDM	China	2	2	2	2	2	1	-	1	4
YSMI	China	2	2	2	2	2	1	2	1	4
W2	Tibet	1	1	2	2	2	1	2	1	4
BR2	Brazil	2	2	2	2	2	1	1	2	5
Hamelin	Australia	1	1	2	2	2	1	2	1	4
Svanhals	CYMMIT	2	2	2	2	2	1	1	1	-
Yambla	China	2	2	2	2	2	1	1	1	2
PTWOOM-2	China	6	2	2	2	2	1	2	1	4
PTWOOM-3	China	6	2	2	2	2	1	2	1	4
PTWOOM-4	China	6	2	2	2	2	1	2	1	4
PTWOOM-5	China	6	2	2	2	2	1	2	1	4
PTWOOM-6	China	6	2	2	2	2	1	2	1	4
PTWOOM-8	China	6	2	2	2	2	1	2	1	4
Pavlovicky	Russia	-	-	-	-	-	-	-	-	-

Table A-2 (continued)

Acession	Origion	U4	U2	U6	HvMATE-21indel	D0	D5	D3	D01R	INT0RR+INT2
Yan90260	China	7	1	3	2	1	1	2	2	1
Haruna nijo	Japan	1	1	3	3	3	2	1	1	1
Honen	Japan	6	1	1	1	1	2	2	2	2
YUQS	China	1	1	3	3	3	2	1	1	1
YWHKSL	China	1	1	1	1	1	2	2	2	1
Yerong	China	1	1	3	3	3	2	1	1	1
Yiwu Erleng	China	6	1	1	1	1	3	1	1	1
Hu93-043	China	-	1	3	3	3	2	1	1	1
Kinu nijo 6	Japan	1	1	3	3	3	2	1	1	1
YYXT	China	1	1	3	3	3	2	1	1	1
Zhepi 2	China	1	1	3	3	3	2	1	1	1
YPSLDM	China	6	1	1	1	1	3	-	-	1
YSMI	China	6	1	1	1	1	3	1	-	1
W2	Tibet	1	1	3	3	3	2	1	1	1
BR2	Brazil	7	1	3	1	1	3	2	2	1
Hamelin	Australia	1	1	3	-	3	2	1	1	1
Svanhals	CYMMIT	7	1	3	2	1	1	2	2	1
Yambla	China	7	1	3	2	1	1	2	2	1
PTWOOM-2	China	6	-	1	1	1	3	2	1	-
PTWOOM-3	China	6	-	1	1	1	3	2	2	-
PTWOOM-4	China	6	-	1	1	1	3	2	2	-
PTWOOM-5	China	6	-	1	1	1	3	2	2	-
PTWOOM-6	China	6	-	1	1	1	3	2	2	-
PTWOOM-8	China	6	-	1	1	1	3	2	2	-
Pavlovicky	Russia	-	-	-	-	-	-	-	-	1

Table A-3 Dendogram subgroups and accession code

Accession name	Code	Subgroup	Accession name	Code	Subgroup	Accession name	Code	Subgroup
Mundah	125	SubG10	Spanish Landrace-316	246	-	YSM3	280	SubG8
Fleet	119	SubG10	Keka	222	-	ZUG403	279	SubG8
Onslow	114	SubG10	M94257001	208	-	ZUG293	278	SubG8
Hindmarsh	113	SubG10	Mundah	298	-	Naso nijo	277	SubG8
Gairde	112	SubG10	CPI-11284-48	288	-	Lixi 143	276	SubG8
Fit39eald	111	SubG10	SYR01	290	-	Aizao 3	275	SubG8
10119	91	SubG10	HAMELIN	326	SubG8	93-3143	274	SubG8
9837	87	SubG10	W2	324	SubG8	HOR13461	268	SubG8
9830	80	SubG10	Zhepi 2	321	SubG8	M94060003	267	SubG8
9827	79	SubG10	YYXT	320	SubG8	H96009015002	266	SubG8
9821	78	SubG10	Kinu nijo 6	319	SubG8	WVB 22	265	SubG8
9816	76	SubG10	Hu93-043	318	SubG8	WVB 9	264	SubG8
9814	75	SubG10	Yerong	316	SubG8	2B03-3882	262	SubG8
9811	74	SubG10	YUQS	314	SubG8	Portuguese Landrace	256	SubG8
9808	71	SubG10	Haruna nijo	312	SubG8	H96009015001	255	SubG8
9801	68	SubG10	Yan89110	310	SubG8	H95032005	254	SubG8
9792	65	SubG10	Xiaojiang	309	SubG8	WVA 24	252	SubG8
9786	63	SubG10	Unicorn	308	SubG8	2B03-3859	251	SubG8
9809	72	-	Gebeina	307	SubG8	2B03-3830	250	SubG8
Spanish - 309d	235	SubG12	Schooner	304	SubG8	Stirling	249	SubG8
Moroccan Landrace	234	SubG12	Tx9425	303	SubG8	Stirling	248	SubG8
Gairdner	225	SubG12	YF374	302	SubG8	HOR12517	245	SubG8
Gairdner	224	SubG12	TF026	297	SubG8	Braemar	244	SubG8
K 70	190	SubG12	Dash	294	SubG8	Braemer	242	SubG8
Precoce du Maroc	199	-	Keel	293	SubG8	WVA 22	241	SubG8
H95052002	197	-	Flagship	292	SubG8	WVA 20	240	SubG8
Cevada de 6 Ordens	186	-	AC Burman	291	SubG8	2B03-3785	239	SubG8
Macquarie	296	-	Clipper	287	SubG8	2B03-3631	238	SubG8
B591	149	-	Barque 73	286	SubG8	Hamelin	237	SubG8
Gairdner	306	-	Numar	284	SubG8	Hamelin	236	SubG8
WVA 18	228	-	CM72	283	SubG8	Wicket	230	SubG8
Boa Fe	210	-	YU6472	281	SubG8	WVA 19	229	SubG8

Table A-3 (continued)

Accession name	Code	Subgroup	Accession name	Code	Subgroup	Accession name	Code	Subgroup
2B03-3604	227	SubG8	B581	145	SubG8	8752	43	SubG8
Cocktail	219	SubG8	B577	144	SubG8	8751	42	SubG8
Waggon	218	SubG8	B575	143	SubG8	8750	41	SubG8
Z118M006M	217	SubG8	B561	142	SubG8	8749	40	SubG8
Z090M066M	216	SubG8	B559	141	SubG8	8744	35	SubG8
HB705	215	SubG8	B557	140	SubG8	8743	34	SubG8
SB03180	214	SubG8	B539	139	SubG8	8742	33	SubG8
Barlis	211	SubG8	B537	138	SubG8	Sahara	299	-
H95011020	209	SubG8	B531	137	SubG8	Flagon	231	-
WVC 3	206	SubG8	B523	136	SubG8	9775	58	-
Z055O012O	205	SubG8	B521	135	SubG8	MAR-86-E1138	257	-
Z052R091S	204	SubG8	B515	134	SubG8	BM9919-90	226	-
TR06106	203	SubG8	XZ54/GG1	130	SubG8	9836	86	-
X112	174	SubG8	XZ76	129	SubG8	9819	77	-
X97	173	SubG8	WABAR2473 (07 WH P1)	128	SubG8	H95042004	196	-
B783	170	SubG8	Baudin	126	SubG8	H95030001	184	-
B771	168	SubG8	Stirling	121	SubG8	Cevada Preta	187	-
B705	164	SubG8	Haningloh	120	SubG8	Vlamingh	261	-
B703	163	SubG8	Doolup	118	SubG8	Vlamingh	260	-
B701	162	SubG8	Flagship	117	SubG8	WVB 35	195	-
B695	161	SubG8	8658	104	SubG8	XZ76	3	-
B685	159	SubG8	8482	93	SubG8	Svanhals	2	-
B671	158	SubG8	9804	69	SubG8	9810	73	-
B659	157	SubG8	9794	66	SubG8	Baudin	201	-
B657	156	SubG8	9791	64	SubG8	H95039003	185	-
B645	154	SubG8	9781	62	SubG8	Spanish Landrace-355	189	-
B627	153	SubG8	9776	59	SubG8	8746	37	-
B617	152	SubG8	8765	56	SubG8	8745	36	-
B615	151	SubG8	8764	55	SubG8	Baudin	200	SubG11
B611	150	SubG8	8760	51	SubG8	WVB 34	194	SubG11
B585	147	SubG8	8759	50	SubG8	Z051R077S	192	SubG11
B583	146	SubG8	8758	49	SubG8	Spanish Landrace-352	188	SubG11

Table A-3 (continued)

Accession name	Code	Subgroup	Accession name	Code	Subgroup	Accession name	Code	Subgroup
WVB 33	183	SubG11	Fitzgerald	213	-	9835	85	-
WVB 29	182	SubG11	Fitzgerald	212	-	9834	84	-
Z035R014S	181	SubG11	Yan90260	311	SubG2	9833	83	-
WABAR2315	273	SubG14	B779	169	SubG2	Spanish Landrace-338c	259	-
WABAR2315	272	SubG14	B751	165	SubG2	WVB 7	253	-
B503	133	SubG14	Buloke	122	SubG2	Honen	313	-
B501	132	SubG14	Cavmen Pavent	109	SubG2	Dayton	295	-
Viamingh(06 NH M4)	124	SubG14	Cavmen	108	SubG2	RGZLL	285	-
Hamelin(09 WH P3)	123	SubG14	8483	94	SubG2	PTWOOM-8	334	SubG13
8756	47	-	10114	89	SubG2	PTWOOM-6	333	SubG13
8755	46	-	9832	82	SubG2	PTWOOM-5	332	SubG13
8754	45	-	9831	81	SubG2	PTWOOM-4	331	SubG13
Skiff	305	SubG1	9440	57	SubG2	PTWOOM-3	330	SubG13
Tipple	207	SubG1	8747	38	SubG2	PTWOOM-2	329	-
Z051R101S	193	SubG1	8587	32	SubG2	B797	171	-
B769	167	SubG1	8586	31	SubG2	B653	155	-
WABAR2473 (06 WH M3)	127	SubG1	8585	30	SubG2	98	5	-
keel	115	SubG1	8581	26	SubG2	X161	178	SubG6
Stirling	1	SubG1	8580	25	SubG2	X153	176	SubG6
Spanish Landrace-333c	247	-	8579	24	SubG2	B587	148	SubG6
4547	15	-	4050	8	SubG2	200 SM1 Barley 3002	110	SubG6
Franklin	301	-	4049	7	SubG2	10118	90	SubG6
X51	172	-	4048	6	SubG2	9807	70	SubG6
Spanish Landrace-349b	271	-	8583	28	-	9799	67	SubG6
Spanish Landrace-336d	258	-	yambla	328	SubG7	9779	61	SubG6
KM 123	179	-	SVANHAL	327	SubG7	8762	53	SubG6
Czech Landrace-243	191	-	10113	88	SubG7	8761	52	SubG6
Rosa	223	-	8582	27	SubG7	8748	39	SubG6
Spanish Landrace-349	270	-	8578	23	SubG7	4812	20	SubG6
H95056005	232	-	8484	22	SubG7	10120	92	-
H95056002	221	-	8475	21	SubG7	Cevada de 2 Ordens	269	-
H95011024	220	-	B759	166	-	B689	160	-

Table A-3 (continued)

Accession name	Code	Subgroup	Accession name	Code	Subgroup
YSMI	323	-	4550	18	SubG5
YPSLDM	322	-	4549	17	SubG5
Yiwu Erleng	317	SubG9	Z034P013Q	263	SubG4
YWHKSL	315	SubG9	Adagio	233	SubG4
CxHKSL	289	SubG9	Z034P116Q	180	SubG4
Noire Maroc	198	SubG9	Maritime	116	SubG4
Sook Awn 86	131	SubG9	8757	48	SubG4
9778	60	SubG9	8584	29	SubG4
8763	54	SubG9	4548	16	SubG4
8661	107	SubG3	H95027004	243	-
8649	99	SubG3	DYSYH	300	-
8648	98	SubG3	X160	177	-
8647	97	SubG3	X117	175	-
8644	96	SubG3	XZ54/GG1	4	-
8643	95	SubG3			
4544	12	SubG3			
4543	11	SubG3			
4444	10	SubG3			
4051	9	SubG3			
CSK-81-556	202	-			
4546	14	-			
4545	13	-			
BR2	325	-			
Brindabella	282	-			
8660	106	-			
8659	105	-			
8655	103	SubG5			
8654	102	SubG5			
8651	101	SubG5			
8650	100	SubG5			
8753	44	SubG5			
4551	19	SubG5			

Table A-4 Accession names, origins and the ten polymorphic markers` gel scoring, - means missing data

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
Stirling	Australia	1	0	1	3	1	1	1	-	1	1
Svanhals	CYMMIT	1	1	1	3	2	1	1	1	1	1
XZ76	China	1	1	2	1	2	-	1	-	1	1
XZ54/GG1	China	1	1	2	1	2	-	1	-	1	1
00098	Australia	1	0	1	3	1	1	1	-	1	1
04048	Australia	1	1	1	3	2	1	1	-	1	1
04049	Australia	1	0	1	3	1	1	1	1	1	1
04050	Australia	2	1	1	3	2	1	2	1	1	1
04051	Australia	2	1	1	2	2	1	2	1	1	1
04444	Australia	2	1	1	2	2	1	2	1	1	1
04543	Switzerland	1	1	1	3	2	1	1	1	1	1
04544	Switzerland	1	1	1	3	2	1	1	1	1	1
04545	Switzerland	1	1	1	3	2	1	1	1	1	1
04546	Switzerland	1	1	1	3	2	1	1	-	1	1
04547	Switzerland	1	1	1	3	2	1	1	-	1	1
04548	Switzerland	1	1	1	3	2	1	1	-	1	1
04549	Switzerland	1	0	1	3	1	1	1	-	1	1
04550	Switzerland	1	1	1	3	2	1	1	1	1	1
04551	Switzerland	1	0	1	3	1	1	1	1	1	1
04812	Pakistan	1	0	1	3	1	1	1	1	1	1
08475	Chile	1	1	1	3	2	1	1	1	1	1
08484	Brazil	1	1	1	3	2	1	1	1	1	1
08578	Brazil	1	1	1	3	2	1	1	1	1	1
08579	Brazil	1	1	1	3	2	1	1	1	1	1
08580	Brazil	1	1	1	3	2	1	1	1	1	1
08581	Brazil	1	1	1	3	2	1	1	1	1	1
08582	Chile	1	0	1	3	1	1	1	1	1	1
08583	Chile	1	0	1	3	1	1	1	-	1	1
08584	Chile	1	1	1	3	2	1	1	1	1	1
08585	Chile	1	1	1	3	2	1	1	1	1	1
08586	Chile	1	1	1	3	2	1	1	1	1	1
08587	Chile	1	1	1	3	2	1	1	1	1	1
08742	Russia	1	1	1	3	2	1	1	1	1	1
08743	Russia	1	0	1	3	1	1	1	1	1	1

Table A-4 (continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
08744	Russia	1	1	1	3	2	1	1	1	1	1
08745	Russia	1	0	1	3	1	1	1	1	1	1
08746	Russia	1	0	1	3	1	1	1	1	1	1
08747	Russia	1	0	1	3	1	1	1	1	1	1
08748	Russia	1	1	1	3	2	1	1	1	1	1
08749	Unknown	1	1	1	3	2	1	1	1	1	1
08750	Unknown	1	1	1	3	2	1	1	1	1	1
08751	Unknown	1	1	1	3	2	1	1	1	1	1
08752	Unknown	1	1	1	3	2	1	1	1	1	1
08753	Unknown	1	1	1	3	2	1	1	1	1	1
08754	Unknown	1	1	1	3	2	1	1	-	1	1
08755	Unknown	1	1	1	3	2	1	1	1	1	1
08756	Unknown	1	1	1	3	2	1	1	1	1	1
08757	Unknown	1	1	1	3	2	1	1	1	1	1
08758	Unknown	1	1	1	3	2	1	1	1	1	1
08759	Unknown	1	1	1	3	2	1	1	1	1	1
08760	Unknown	1	1	1	3	2	1	1	1	1	1
08761	Unknown	1	1	1	3	2	1	1	1	1	1
08762	Unknown	1	1	1	3	2	1	1	1	1	1
08763	Unknown	1	1	1	3	2	1	1	1	1	1
08764	Unknown	1	1	-	3	2	1	1	1	1	1
08765	Unknown	1	1	1	3	2	1	1	1	1	1
09440	Germany	1	1	1	3	2	1	1	-	1	1
09775	Spain	1	0	1	3	1	1	1	1	1	1
09776	Spain	1	0	1	3	1	1	1	1	1	1
09778	Morocco	1	1	1	3	2	1	1	1	1	1
09779	Spain	1	1	1	3	2	1	1	1	1	1
09781	Portugal	1	1	1	3	2	1	1	1	1	1
09786	Morocco	1	0	1	3	1	1	1	1	1	1
09791	Morocco	1	0	1	3	1	1	1	1	1	1
09792	Morocco	1	0	1	3	1	1	1	1	1	1
09794	Spain	1	0	1	3	1	1	1	1	1	1
09799	Portugal	1	1	1	3	2	1	1	1	1	1
09801	Portugal	1	0	1	3	1	1	1	1	1	1

Table A-4 (continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
09804	Portugal	1	1	1	3	2	1	1	-	1	1
09807	Morocco	1	1	1	3	2	1	-	1	1	1
09808	Morocco	1	0	1	3	1	1	1	1	1	1
09809	Portugal	1	1	1	3	2	1	1	1	1	1
09810	Morocco	2	1	1	3	2	1	2	1	1	1
09811	Morocco	1	0	1	3	1	1	1	1	1	1
09814	Spain	1	0	1	3	1	1	1	1	1	1
09816	Spain	1	0	1	3	1	1	1	-	1	1
09819	Spain	1	0	1	3	1	1	1	1	1	1
09821	Morocco	1	0	1	3	1	1	1	1	1	1
09827	Spain	1	0	1	3	1	1	1	1	1	1
09830	Spain	1	0	1	3	1	1	1	1	1	1
09831	Spain	1	0	1	3	1	1	1	1	1	1
09832	Spain	1	0	1	3	1	1	1	1	1	1
09833	Spain	3	0	1	3	1	1	1	1	1	1
09834	Spain	1	0	1	3	1	1	1	1	1	1
09835	Spain	1	0	1	3	1	1	1	1	1	1
09836	Spain	1	1	1	3	2	1	1	1	1	1
09837	Spain	1	0	1	3	1	1	1	1	1	1
10113	Brazil	1	1	1	3	2	1	1	1	1	1
10114	Brazil	3	0	1	3	1	1	1	1	1	1
10118	America	1	0	1	3	1	1	1	1	1	1
10119	America	1	0	1	3	1	1	1	1	1	1
10120	America	1	0	1	3	1	1	1	1	1	1
8482	Unknown	1	1	1	3	2	1	1	1	1	1
8483	Unknown	1	1	1	3	2	1	1	1	1	1
8643	Unknown	1	1	1	3	2	1	1	1	1	1
8644	Unknown	1	1	1	3	2	1	1	1	1	1
8647	Unknown	1	1	1	3	2	1	1	1	1	1
8648	Unknown	1	1	1	3	2	1	1	1	1	1
8649	Unknown	1	1	1	3	2	1	1	1	1	1
8650	Unknown	1	1	1	3	2	1	1	1	1	1
8651	Unknown	1	1	1	3	2	1	1	1	1	1
8654	Unknown	1	1	1	3	2	1	1	1	1	1

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
8655	Unknown	1	1	1	3	2	1	1	-	1	1
8658	Unknown	1	0	1	3	1	1	1	1	1	1
8659	Unknown	1	1	1	3	2	1	1	1	1	1
8660	Unknown	1	1	1	3	2	1	1	1	1	1
8661	Unknown	1	1	1	3	2	1	1	1	1	1
Cavmen	Australia	1	1	1	3	2	1	1	-	1	1
Cavmen Pavent	Australia	1	1	1	3	2	1	1	-	1	1
200 SM1 Barley 3002	Unknown	1	0	1	3	1	1	1	1	1	1
Fit39eald	Australia	1	1	1	3	2	1	1	1	1	1
Gairde	Australia	1	1	1	3	2	1	1	1	1	1
Hindmarsh	Australia	1	0	1	3	1	1	1	1	1	1
Onslow	Australia	1	1	1	3	2	1	1	1	1	1
keel	Australia	1	0	1	3	1	1	1	1	1	1
Maritime	Australia	1	0	1	3	1	1	1	1	1	1
Flagship	Australia	2	1	1	3	2	1	2	1	1	1
Doolup	Australia	1	0	1	3	1	1	1	1	1	1
Fleet	Australia	1	0	1	3	1	1	1	1	1	1
Haningloh	Canada	1	1	1	3	2	1	1	1	1	1
Stirling	Australia	1	0	1	3	1	1	1	1	1	1
Buloke	Australia	1	0	1	3	1	1	1	-	1	1
Hamelin(09 WH P3)	Australia	1	0	1	3	1	1	1	1	1	1
Viamingh(06 NH M4)	Australia	1	0	1	3	1	1	1	1	1	1
Mundah	Australia	1	0	1	3	1	1	1	1	1	1
Baudin	Australia	1	0	1	3	1	1	1	1	1	1
WABAR247 3 (06 WH M3)	Australia	1	0	1	3	1	1	1	1	1	1
WABAR247 3(07 WH P1)	Australia	1	0	1	3	1	1	1	1	1	1
XZ76	China	1	1	2	1	2	-	1	2	2	1
XZ54/GG1	China	1	1	2	1	2	-	1	2	2	1
Sook Awn 86	America	1	0	1	3	1	1	1	1	1	1
B501	China	1	1	1	-	2	1	1	-	1	1
B503	China	1	1	1	3	2	1	1	1	1	1
B515	China	1	1	1	3	2	1	1	1	1	1
B521	China	1	1	1	3	2	1	1	1	1	1
B523	China	1	1	1	3	-	1	1	1	1	1

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
B531	China	1	1	1	3	2	1	1	1	1	1
B537	China	1	1	1	3	2	1	1	1	1	1
B539	China	1	1	1	3	2	1	1	1	1	1
B557	China	1	1	1	3	2	1	1	1	1	1
B559	China	1	1	1	3	2	1	1	1	1	1
B561	China	1	0	1	3	1	1	1	-	1	1
B575	China	1	1	1	3	2	1	1	1	1	1
B577	China	1	1	1	3	2	1	1	1	1	1
B581	China	1	1	1	3	2	1	1	1	1	1
B583	China	1	1	1	3	2	1	1	1	1	1
B585	China	1	1	1	3	2	1	1	1	1	1
B587	China	1	0	1	3	1	1	1	1	1	1
B591	China	1	0	1	3	1	1	1	1	1	1
B611	China	1	0	1	3	1	1	1	1	1	1
B615	China	1	1	1	3	2	1	1	1	1	1
B617	China	1	1	1	3	2	1	1	1	1	1
B627	China	1	1	1	3	2	1	1	1	1	1
B645	China	1	0	1	3	1	1	1	-	1	1
B653	China	1	0	1	3	1	1	1	1	1	1
B657	China	1	1	1	3	2	-	1	1	1	1
B659	China	1	1	1	3	2	1	1	1	1	1
B671	China	1	1	1	3	2	1	1	1	1	1
B685	China	1	1	1	3	2	1	1	1	1	1
B689	China	1	0	1	3	1	1	1	1	1	1
B695	China	1	1	1	3	2	1	1	1	1	1
B701	China	1	1	1	3	2	1	1	1	1	1
B703	China	1	1	1	3	2	1	1	1	1	1
B705	China	1	0	1	3	1	1	1	-	1	1
B751	China	1	0	1	3	1	1	1	-	1	1
B759	China	1	0	1	3	1	1	1	1	1	1
B769	China	1	1	1	3	2	1	1	1	1	1
B771	China	1	1	1	3	2	1	1	1	1	1
B779	China	1	0	1	3	1	1	1	1	1	1
B783	China	1	0	1	3	1	1	1	1	1	1

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
B797	China	1	0	1	3	1	1	1	1	1	1
X51	China	1	1	2	3	2	-	1	2	2	1
X97	China	1	0	1	3	1	1	1	1	1	1
X112	China	1	1	2	1	2	1	1	2	2	1
X117	China	1	0	1	3	1	1	1	1	1	1
X153	China	1	1	1	3	2	1	1	1	1	1
X160	China	1	0	1	3	1	1	1	1	1	1
X161	China	1	1	1	1	2	1	1	1	1	1
KM 123	China	1	1	1	3	2	1	1	1	1	1
Z034P116Q	China	1	1	1	3	2	1	1	1	1	1
Z035R014S	China	1	1	1	3	2	1	1	1	1	1
WVB 29	Unknown	1	1	1	3	2	1	1	1	1	1
WVB 33	Unknown	1	1	1	3	1	1	1	1	1	3
H95030001	Canada	1	1	1	3	2	1	1	1	1	1
H95039003	Canada	1	1	1	3	2	1	1	1	1	1
Cevada de 6 Ordens	Portugal	1	0	1	3	1	1	1	-	1	1
Cevada Pret	Portugal	-	-	1	3	2	1	1	1	1	1
Spanish Landrace-352	Spain	1	0	1	3	2	1	1	1	1	1
Spanish Landrace-355	Spain	1	0	1	3	-	1	1	1	1	1
K 70	Unknown	1	1	1	3	1	1	1	1	1	1
Czech Landrace-243	Czech	1	1	1	3	2	1	1	1	1	1
Z051R077S	China	1	1	1	3	2	1	1	1	1	1
Z051R101S	China	1	1	1	3	2	1	1	1	1	1
WVB 34	Unknown	1	0	1	3	1	1	1	1	1	1
WVB 35	Unknown	1	1	1	3	2	1	1	1	1	1
H95042004	Canada	1	1	1	3	2	1	1	1	1	1
H95052002	Canada	1	-	1	3	1	1	1	1	1	1
Noire Maroc	Morocco	1	1	1	3	2	1	1	1	1	1
Precoce du Maroc	Morocco	-	1	1	3	-	1	-	1	1	-
Baudin	Australia	1	-	1	3	1	1	1	1	1	1
Baudin	Australia	1	0	1	3	1	1	1	1	1	1
CSK-81-556	Unknown	1	1	1	-	2	1	1	1	1	1

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
TR06106	Canada	1	1	1	3	2	1	1	1	1	1
Z052R091S	China	1	1	1	3	2	1	1	1	1	1
Z055O012O	China	-	1	1	3	2	1	1	1	1	1
WVC 3	Unknown	1	1	1	3	2	1	1	1	1	3
Tipple	The United Kingdom	1	1	1	3	2	1	1	1	1	1
M94257001	Canada	1	0	1	3	2	1	1	1	1	1
H95011020	Canada	1	1	1	3	2	1	1	1	1	1
Boa Fe	Portugal	1	1	1	3	2	1	1	1	1	1
Barlis	Morocco	1	-	1	3	2	1	2	1	1	1
Fitzgerald	Australia	1	1	1	3	2	1	1	1	1	1
Fitzgerald	Australia	1	1	1	3	2	1	1	1	1	1
SB03180	Canada	1	1	1	3	2	1	1	1	1	1
HB705	Canada	1	1	1	3	2	1	1	1	1	1
Z090M066M	China	1	1	1	3	2	1	1	1	1	1
Z118M006M	China	1	1	1	3	2	1	1	1	1	1
Waggon	The United Kingdom	1	1	1	3	2	1	1	1	1	1
Cocktail	The European Union	1	1	1	3	2	1	1	1	1	1
H95011024	Canada	1	1	1	3	2	1	1	1	1	1
H95056002	Canada	1	1	1	3	2	1	1	1	1	1
Keka	Spain	1	0	1	3	1	1	1	1	1	1
Rosa	Unknown	-	0	1	3	1	1	1	1	1	1
Gairdner	Australia	1	1	1	3	2	1	1	1	1	1
Gairdner	Australia	1	1	1	3	2	1	1	1	1	1
BM9919-90	Canada	1	1	1	3	1	1	1	1	1	3
2B03-3604	Unknown	1	1	1	3	2	1	1	1	1	1
WVA 18	Unknown	2	1	1	2	2	1	2	1	1	1
WVA 19	Unknown	2	1	1	2	2	1	2	1	1	1
Wicket	The United Kingdom	1	1	1	3	2	1	1	1	1	1
Flagon	Unknown	1	0	1	3	1	1	1	1	1	1
H95056005	Canada	1	1	1	3	2	1	1	1	1	1
Adagio	Unknown	1	1	1	3	2	1	1	1	1	1
Moroccan Landrace	Morocco	1	0	1	3	1	1	1	1	1	1

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
Spanish - 309d	Spain	1	0	1	3	1	1	1	1	1	1
Hamelin	Australia	1	0	1	3	1	1	1	1	1	1
Hamelin	Australia	1	0	1	3	1	1	1	1	1	1
2B03-3631	Unknown	1	1	1	3	2	1	1	1	1	1
2B03-3785	Unknown	1	1	1	3	2	1	1	1	1	1
WVA 20	Unknown	1	1	1	3	2	1	1	1	1	1
WVA 22	Unknown	1	1	1	3	2	1	1	1	1	1
Braemer	Germany	1	1	1	3	2	1	1	1	1	1
H95027004	Canada	1	1	1	3	2	1	1	1	1	1
Braemar	Germany	1	1	1	3	2	1	1	1	1	1
HOR12517	Spain	1	0	1	3	-	1	1	1	1	1
Spanish Landrace-316	Spain	1	0	1	3	1	1	1	1	1	1
Spanish Landrace-333c	Spain	1	0	1	3	1	1	1	-	1	1
Stirling	Australia	1	0	1	3	1	1	1	1	1	1
Stirling	Australia	1	0	1	3	1	1	1	1	1	1
2B03-3830	Unknown	1	1	1	3	2	1	1	1	1	1
2B03-3859	Unknown	1	1	1	3	2	1	1	1	1	1
WVA 24	Unknown	1	1	1	3	2	1	1	1	1	1
WVB 7	Unknown	1	1	1	3	2	1	1	-	1	1
H95032005	Canada	1	1	1	3	2	1	1	1	1	1
H96009015(01	Canada	1	1	1	3	2	1	1	1	1	1
Portuguese Landrace	Portugal	1	1	1	3	2	1	1	1	1	1
MAR-86-E1138	Unknown	1	0	1	3	1	1	1	1	1	1
Spanish Landrace-336d	Spain	1	0	1	3	1	1	1	-	1	1
Spanish Landrace-338c	Spain	1	0	1	3	1	1	1	1	1	1
Vlamingh	Australia	1	0	1	3	1	1	1	1	1	1
Vlamingh	Australia	1	0	1	3	1	1	1	1	1	1
2B03-3882	Unknown	1	1	1	3	2	1	1	1	1	1
Z034P013Q	China	1	1	1	3	2	1	1	1	1	1
WVB 9	Unknown	1	1	1	3	2	1	3	1	1	3

Table A-4(continued)

Accession	Origin	UA21	UA34	XCA10	CA15	XCA16	XCA14	UA27	XCA13	CA12	UA37
Sahara	Africa	1	0	1	3	1	1	1	1	1	1
DYSYH	China	1	0	1	3	1	1	1	1	1	1
Franklin	Australia	1	1	1	3	2	1	1	1	1	1
YF374	China	1	0	1	3	1	1	1	1	1	1
Tx9425	China	1	1	1	3	2	1	1	1	1	1
Schooner	Australia	2	1	1	2	2	1	2	-	1	1
Skiff	Australia	1	0	1	3	1	1	1	1	1	1
Gairdner	Australia	1	1	1	3	2	1	1	1	1	1
Gebeina	Germany	1	0	1	3	1	1	1	1	1	1
Unicorn	Japan	1	1	1	3	2	1	1	1	1	1
Xiaojiang	China	1	0	1	3	1	1	1	1	1	1
Yan89110	China	1	1	1	3	2	1	1	1	1	1
Yan90260	China	1	1	1	3	2	1	1	-	1	1
Haruna nijo	Japan	1	1	1	3	2	1	1	1	1	1
Honen	Japan	1	0	1	3	1	1	1	1	1	1
YUQS	China	1	0	1	3	1	1	1	1	1	1
YWHKSL	China	1	0	1	3	1	1	1	1	1	1
Yerong	China	1	1	1	3	2	1	1	1	1	1
Yiwu Erleng	China	1	1	1	3	2	1	1	1	1	1
Hu93-043	China	1	1	1	3	2	1	1	1	1	1
Kinu nijo 6	Japan	1	1	1	3	2	1	1	1	1	1
YYXT	China	1	1	1	3	2	1	1	1	1	1
Zhepi 2	China	1	1	1	3	2	1	1	1	1	1
YPSLDM	China	1	0	1	3	1	1	1	1	1	1
YSMI	China	1	0	1	3	1	1	1	1	1	1
W2	Tibet	1	0	1	3	1	1	1	1	1	1
BR2	Brazil	1	1	1	3	2	1	1	1	1	1
Hamelin	Australia	1	0	1	3	1	1	1	1	1	1
Svanhals	CYMMIT	1	1	1	3	2	1	1	1	1	1
Yambla	China	1	1	1	3	2	1	1	1	1	1
PTWOOM-4	China	1	0	1	3	1	1	1	1	1	1
PTWOOM-5	China	1	0	1	3	1	1	1	1	1	1
PTWOOM-6	China	1	0	1	3	1	1	1	1	1	1
PTWOOM-8	China	1	0	1	3	1	1	1	1	1	1
Pavlovicky	Russia	1	0	1	3	1	-	1	-	1	1